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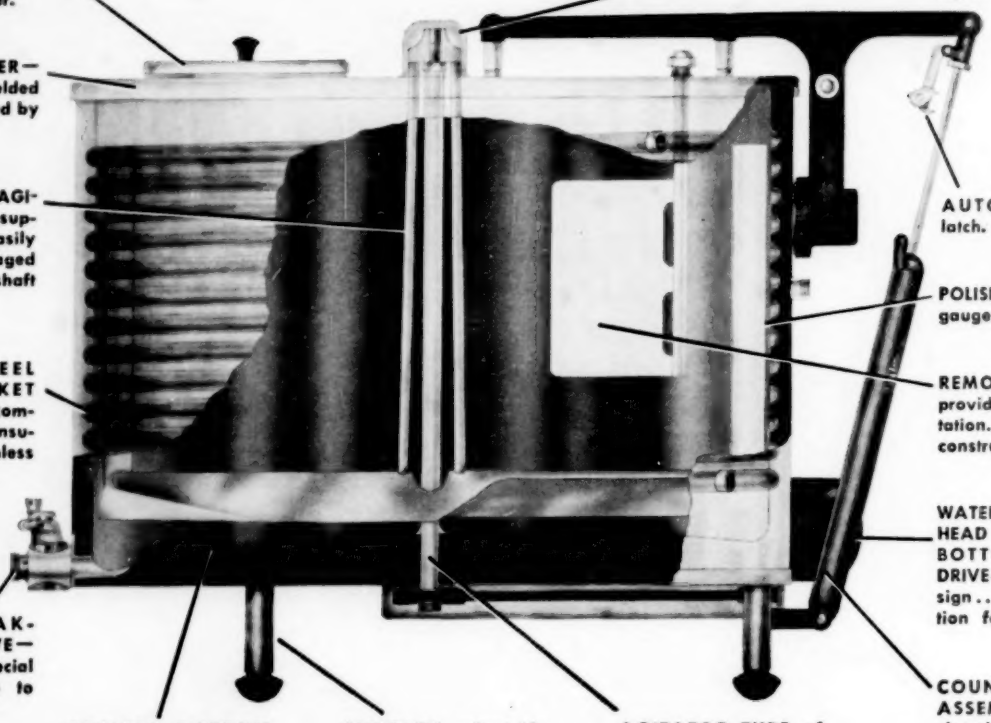

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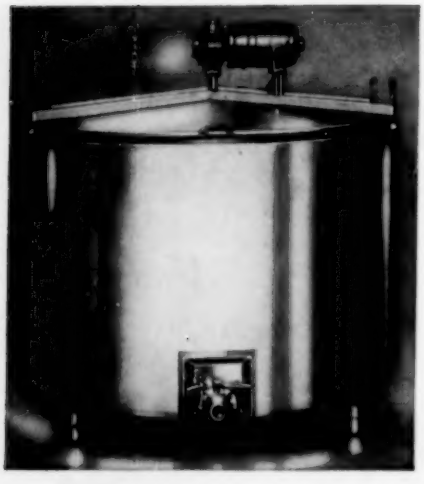

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Quality and Nutritive Properties of Different Types of Commercially Cured Hams. 1. Curing Methods and Chemical Composition[a,b]

M. D. FIELDS AND C. F. DUNKER

Animal Husbandry Division, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Maryland[c]

(Manuscript received April 26, 1952)

The typical methods used in curing meat by commercial packing companies are reviewed. Experimental data and a discussion of chemical composition of hams cured by these typical methods are given.

Approximately 10.5 billion pounds of hog carcass meat are obtained annually from slaughtering operations in the United States by commercial meat packing plants under federal inspection.[d] Of this meat, approximately 3.5 billion pounds are cured. According to the Meat Inspection Division of the Bureau of Animal Industry *(9)*, this consists in large part of hams, picnic shoulders, and bacons processed by 900 packing houses.

Although there is some scattered information available regarding curing methods and studies of ham composition, there is lacking in the literature data on the latter in relation to curing methods. As a part of a comprehensive study of processing in relation to preservation of quality and nutritive value of meats, such information has been obtained and is presented here.

This paper includes the results of a survey made to determine the most generally used commercial methods of curing hams. Based on the results of this survey, cured hams proudced by these typical methods were obtained, and a study of their chemical composition was made. It is planned in a later paper to present the data that have been obtained on organoleptic evaluation, bacteriological characteristics, content of certain vitamins, and the biological value of the protein. In subsequent work the typical methods of curing will be duplicated under controlled experimental conditions with the use of meats from animals of known breeding, feeding, and other factors. Hams so treated will be analyzed for the same components as were the typical samples obtained from the commercial plants. Finally, in light of the information obtained on products resulting from "typical" methods, studies will be conducted on modifications in such methods to determine their effects on quality and nutritive values.

SURVEY OF COMMERCIAL CURING METHODS

In determining the typical methods of commercial ham curing, references were made to Pork Operations *(11)*, Dillon *(3)*, Jensen *(7)*, and Modern Curing Practices *(1)*. A summary was prepared from these references that was considered to represent the typical methods of ham curing used by the meat packing industry. Copies of these typical methods of commercial meat curing were sent to the National Independent Meat Packers Association and the American Meat Institute, who distributed them among representative members of the respective associations for critical evaluation. The comments and suggestions received from these packers were used to revise the tentative typical methods indicated above. These revisions were again sent to the American Meat Institute and the National Independent Meat Packers Association for their final approval.

Results of this survey showed that there are four predominant methods of ham curing used by commercial packers. These methods may be identified as: I, sweet pickle, quick-cured, tendered; II, sweet pickle, quick-cured, ready-to-eat; III, sweet pickle, long-cured; and IV, the dry-cured Smithfield process.

After determining to the best of our ability just what were considered the typical methods of commercially curing hams, representative samples of each type of curing (I, II, III, and IV) were obtained for determination of chemical composition. Where possible, 5 samples of each type of cured ham were obtained from a large, from a small, and from an intermediate-sized packing company. By this method, we hoped not only to compare one type of curing with another, but also to determine whether any significant differences existed in composition of hams cured by the same methods, but by different packing companies.

Curing Methods

The detailed description of each of the types of curing which follows is a composite picture of the methods in use. In general the packing companies follow the methods as outlined, but may deviate somewhat at different stages of processing. All of them have special modifications which in their minds give their product a distinctive appeal to the public. Hence, the typical methods as outlined may not apply in every detail to any packing company.

Type I. Sweet pickle, quick-cured, tendered hams. There are 3 steps in preparing this type of cured ham: first, pumping; next, curing in cover pickle; and finally, the smoking process. Pumping can be either by artery or by spray methods, most packers preferring the former, since this is quicker and when properly carried out assures a uniform distribution of the pump pickle. A reliable formula for artery pump pickle, used with minor variations by most packing companies is to prepare a 70° salometer pickle at 38° F. (3.3° C.) containing, per 100 gallons of pickle, 22 pounds of cane sugar, 1.5 pounds of sodium nitrite, 1 pound of sodium nitrate, and sufficient salt to obtain the desired salometer reading. Spray pump pickle contains the same amounts of nitrite, nitrate, and sugar but has a final salometer reading of 80°. These hams were pumped to an 8 to 10% net weight increase. The second step in the curing process is to brine cure

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[a] Report of study made under the Research and Marketing Act of 1946.

[b] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Mich., June 9, 1952.

[c] H. Slofsky made the statistical analysis of the data; W. Kauffman determined the proximate composition of the meat; and E. Birmingham assisted in the preparation of the samples in part of the chemical work.

[d] Information derived from weekly and monthly estimates of meat production, released by the Livestock Branch, Production and Marketing Administration, November, 1951.

the pumped hams by placing them into a cover pickle for an average of 10 days at 38° F. (3.3° C.). Many operators cure for only 4 days, some cure as long as 15 days, others cure for 8 days and then let the hams "drain" at 38° F. (3.3° C.) on wooden bins for an additional 8 days. Occasionally a curing temperature as high as 42° F. (5.5° C.) may be used. A reliable formula for cover pickle used by many packers, with slight modifications, is to prepare a pickle having a salometer of from 65-70° at 38° F. (3.3° C.) with each 100 gallons containing 13 pounds of sugar, 1.5 pounds of sodium nitrite, 1.5 pounds of sodium nitrate, and sufficient salt to give the desired final salometer reading. About 5.5 gallons of cover pickle are used for each 100 pounds of meat cured.

In the final step after curing, the hams were washed in a spray of warm water. Then they were drained, dried, and smoked. Uusually 16 to 18 hours were required in the smokehouse to bring the internal temperature up to 142° F. (61° C.) and then this temperature was maintained for 1 hour.* [A good practice is to start the smokehouse at 135° F. (57° C.) and then raise to about 170° F. (76.7° C.) at the rate of 5° F. (2.8° C.) per hour. Once the internal temperature approaches 140° F. (60° C.) the house temperature is lowered to about 145° F. (62.8° C.). Dampers are open for the first few hours.] The hams were then cooled by refrigeration to 50° F. (10° C.) and stored at this temperature.

Type II. The sweet pickle, quick-cured, ready-to-eat hams were processed similarly to the tendered hams except that they were smoked to at least 155° F. (68.3° C.) internal temperature. Many packing plants smoke to internal temperatures of 160° F. (71° C.) and 165° F. (74° C.). The hams were then cooled to 40° F. (4.4° C.) and stored at this temperature.

Type III. The sweet pickle, long-cured hams were spray pumped with a brine solution to produce an 8 to 10% weight increase and then immersed in a cover brine at 38° F. (3.3° C.) and cured for 3 to 4 days per pound of meat. The cover and pumping solutions were prepared in the same way as were those used for the tendered hams. The long brine-cured hams were overhauled after 3, 6, 10, and 15 days in cure. After curing, the hams were soaked in 70° F. (21° C.) water for 3.5 minutes per day in cure. They were then drained, dried, and smoked for a period of 20 to 26 hours to an internal temperature of about 120° F. (49° C.) and stored at 65° F. (18.3° C.) or lower.

Type IV. The Smithfield type of hams, reported to be obtained from peanut-fed hogs, were prepared for curing by sprinkling with sodium nitrate until the hams were white, using 3 to 4 pounds sodium nitrate per 1000 pounds of meat, and then rubbing immediately with fine salt covering the entire surface. The hams were piled 3 hams deep and cured at a temperature of 40° F. (4.4° C.) to 45° F. (7.2° C.). After 3 days of curing the piles of hams were "broken," rubbed again with salt and permitted to cure again for 1 day per pound of ham. After curing, the hams were washed in warm water, drained, and when nearly dry, the entire surface was rubbed with fine black pepper. They were then smoked at a low temperature of 80° F. (26.7° C.) to 110° F. (43.3° C.) for 30 to 40 days, usually smoked only 12 out of 24 hours daily. After smoking, the hams were aged at ambient temperatures for 7 to 18 months.

Chemical Composition

Materials and procedure. Where possible, 5 samples of cured, smoked hams, processed as nearly as possible according to the typical methods, were obtained from a small, a large, and an intermediate sized packing company. This made a total of 15 samples of each type of commercial cure.

Shortly after receipt of the smoked hams representing the different processes, they were analyzed for proximate composition, fat and protein breakdown, chloride, and pH. For comparison fresh hams were analyzed for the same components. Proximate analyses were made on the lean tissue for moisture, protein, ether extract, and ash content by the methods of the Association of Official Agricultural Chemists *(2)*, and for chlorides by the Volhard method as described by Kerr *(8)*. Chlorides are reported in terms of percentage of sodium chloride. Soluble nitrogen and pH were determined on 5% sodium chloride extracts in which 250 ml. of 5% sodium chloride solution was blended with 12.5 g. of lean meat. Non-protein nitrogen (tungstic acid method) was obtained by the procedure of Hiller and Van Slyke *(5)*. The results for nitrogen were calculated as percentage of total nitrogen. The percentage of free fatty acids, expressed as oleic acid content of the fat, and the peroxide value as milliequivalents per 1000 g. of fat, were determined by the methods of Rockwood, Ramsbottom, and Mehlenbacher *(10)* and as reported at the meeting of the International Commission on Fats and Oils *(6)*.

* Meat Inspection Division requirement is a minimum internal temperature of 137° F. (58.2° C.).

RESULTS AND DISCUSSIONS

The results of the analyses of 15 samples each of fresh, tendered (Type I), ready-to-eat (Type II), long brine cured (Type III), and Smithfield hams (Type IV) are shown in Table 1, and the averages are also presented graphically in Figure 1. Hereafter in the discussion, these types of hams will be referred to, respectively, as Type I, II, III, or IV. Table 1 shows the average chemical analysis of each of the five samples from each company, as well as a combined average for each 15 hams of each type of cure. From Table 1 can be seen by comparison the values for each chemical entity in relation to its cure by a large, a small, or an intermediate-sized packing company. Exceptions will be noted in the case of Type III hams where product from a company of intermediate size was not available and hams were therefore obtained from one large and two small companies, and in the case of Type IV hams where all three processors were classed as intermediate. As a matter of convenience, the average of the chemical values for each type of cure (15 samples) has been graphed as shown in Figure 1.

A Comparison of Hams Cured by Different Companies

The data in Table 1 showed that there were certain interesting similarities and differences between companies using the same type of cure.

Type I. There appeared to be no marked differences in any of the chemical components in this group of hams as cured by large, intermediate, and small packing companies.

Type II. For the most part the three companies produced hams which were quite similar in composition. However, the product of Company C (small) was higher in moisture and lower in fat content than were the hams from Companies A (large) and B (intermediate). This was probably due to its initial composition (fresh ham). On the other hand, Company A produced hams with over 2% more sodium chloride than did the other companies, undoubtedly due to the use of stronger pumping or cover brines.

Type III. The only difference observed in this type of cure was that Company D (small) showed the highest soluble nitrogen content. This set of hams was in storage after processing for a comparatively longer period of time, which may have been responsible for this higher value.

Type IV. Athough there were noticeable differences in moisture, fat, salt, free fatty acids, and peroxide values among the hams from the several companies, these differences are believed to have been largely due to

TABLE 1

Chemical analyses of cured commercial and fresh hams [1]

Types of cure	Moisture	Protein (N × 6.25)	Fat	Ash	Sodium chloride	Soluble nitrogen	Non-protein nitrogen	Soluble protein nitrogen	Free fatty acids (as oleic acid)	Peroxide value, m.e.q. per 1000 g.	pH
I	%	%	%	%	%	%	%	%	%		
A, large	67.8	19.6	7.98	4.58	4.25	13.5	11.5	1.96	0.81	0.75	6.03
B, intermediate	68.9	20.9	6.18	3.80	3.36	13.2	12.0	1.24	0.33	0.56	6.17
D, small	66.9	21.1	7.44	4.95	4.48	12.5	11.9	0.63	0.62	1.01	6.30
Average	67.9	20.5	7.20	4.44	4.03	13.1	11.8	1.28	0.59	0.77	6.17
II											
A, large	67.0	20.4	6.77	5.60	5.02	11.3	10.1	1.18	0.50	1.16	6.15
B, intermediate	67.6	21.2	7.55	3.41	2.96	11.5[2]	11.2[2]	0.29	0.31	0.62	6.04
C, small	71.2	20.9	4.94	3.29	2.89	10.0	9.7[2]	0.35	0.42	1.21	6.27
Average	68.6	20.8	6.42	4.10	3.62	10.9	10.3	0.61	0.41	1.00	6.15
III											
A, large	66.4	21.6	6.20	5.89	5.47	11.8	11.5	0.29	0.75	1.37	6.13
D, small	67.6	20.5	6.36	5.76	4.96	16.3	12.0	4.28	0.53	1.95	6.07
E, small	67.3	21.9	5.38	5.69	5.19	10.5	10.4	0.11	0.66	0.92	6.25
Average	67.1	21.3	5.98	5.78	5.21	12.9	11.3	1.56	0.64	1.41	6.15
IV											
F, intermediate	49.8	28.0	11.1	9.95	9.40	33.9	26.0	7.83	10.88	3.91	5.92
G, intermediate	53.2	27.7	9.4	8.53	7.84	34.8	27.6	7.21	8.60	4.60	5.90
H, intermediate	49.9	27.4	13.1	8.49	7.90	36.6	27.0	9.60	8.35	6.22	5.93
Average	51.0	27.7	11.2	8.99	8.38	35.1	26.9	8.15	9.28	4.91	5.92
Fresh											
Average	73.7	21.4	3.77	1.10	0.10	31.8	12.3	19.6	0.48	1.07	5.94

[1] Figures indicated are averages based on samples of five hams from each processing company represented by symbols A, B, C, D, E, F, G, and H and fifteen hams comprising the fresh sample.

[2] Average of 4 hams.

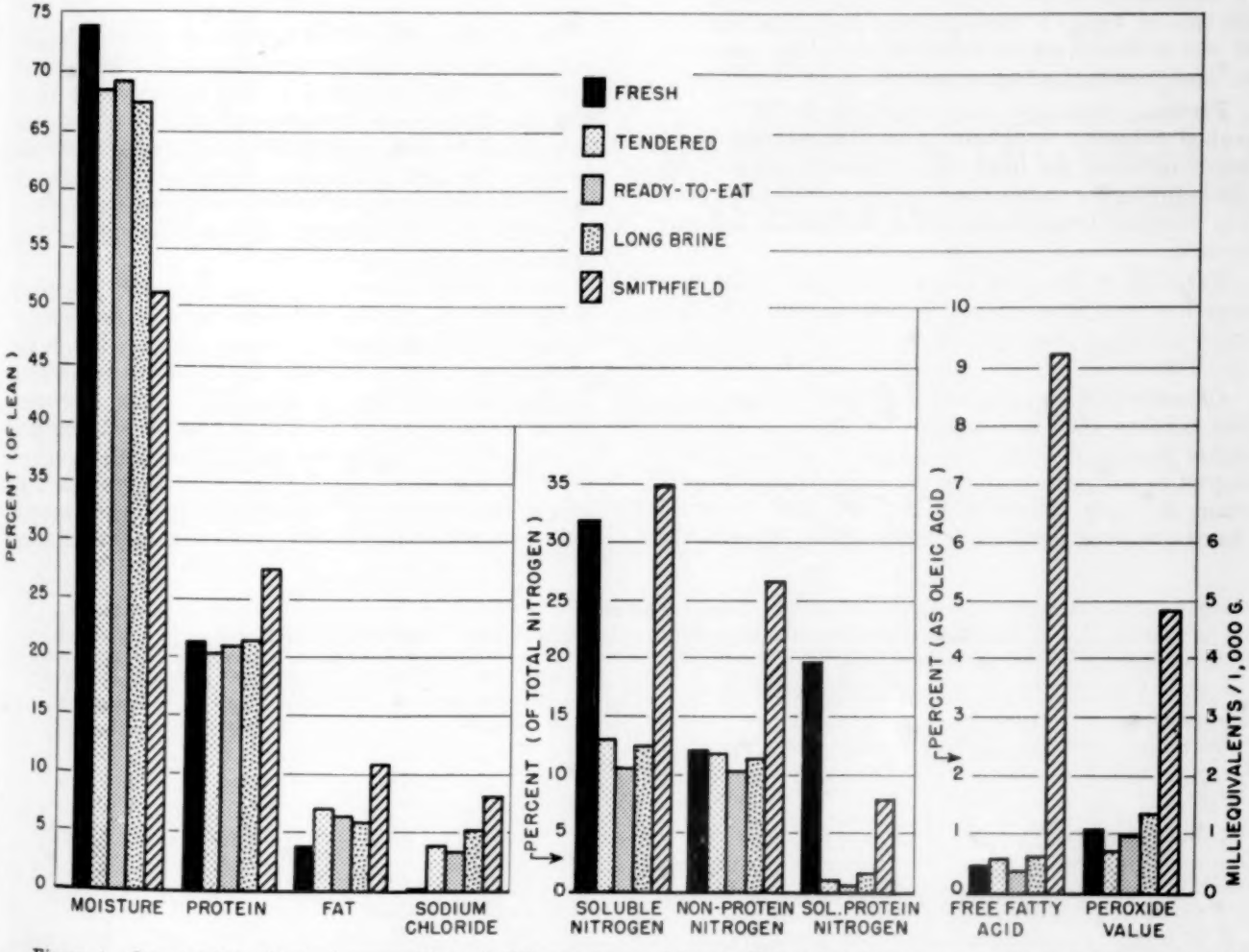

Figure 1. Comparative chemical composition of different types of hams. Values based on averages obtained in analyzing 15 hams of each type.

variations in storage conditions. Hams produced by Company F were in storage 17 months; Company G, 14 months; and Company H, 12 months.

A Comparative Study of Hams Cured by Typical Methods

Table 1 shows average values and Figure 1 gives a graphic summarization of the various types of cure in relation to each other and to fresh hams.

An analysis of the data was made to determine whether the observed differences were statistically significant. Comparisons of the results were determined by the "t" value method, as outlined by Fisher *(4)*. In the following discussion of results, as observed in Table 2, those differences in composition were judged to be valid which were significant at the 1 and 5% levels. With further reference to the statistical analysis, it will be noted that differences in each component shown in Table 2 were determined on each type of cure (15 samples each), rather than for each company (5 samples each).

Moisture. All types of cured hams contained significantly less moisture than fresh hams. The brine-cured hams were quite similar to each other but the Type IV hams contained much less moisture than the brine-cured hams. These differences in moisture content were associated with the variations in the amount of dehydration which occurred during processing. In the case of Type IV hams the very low value was due in part to their being dry cured and to a large extent by the long smoking and aging periods.

Protein. Although there appeared to be no important difference in protein content between any of the brine cured and the fresh hams, Type IV showed significantly higher values than the others. This increase was inversely proportional to the decrease in moisture content.

Fat. All of the cured hams were higher in fat than were the fresh hams. Type IV contained more fat than the brine-cured hams due to considerable dehydration which occurred.

Chloride. Of the ten possible differences in salt content between the hams as seen in Table 2, all were highly significant except one (Type I and II). With respect to the relation of the brine-cured hams to each other, the only difference found was that Type III hams contained significantly more sodium chloride than Types I and II. It is apparent that the higher salt content of the long brine-cured hams was due to the stronger pickle (80° salometer) used in spray pumping as well as the long curing period (3-4 days per pound). It is apparent that the Smithfield hams should have a higher salt content than the other types of cured hams because a stronger concentration of salt was used in processing, and more dehydration occurred as a result of the lengthy smoking and aging periods.

Soluble nitrogen, non-protein nitrogen, and soluble protein nitrogen. The relative degree of protein breakdown is clearly shown graphically in Figure 1, and by any significant differences in Table 2. The soluble nitrogen content of the brine-cured hams was lower and of Type IV hams was higher than the fresh hams. There was no significant difference between Types I and III; however, these types had more soluble nitrogen than Type II hams. The Type IV hams on the other hand had markedly more soluble nitrogen than the brine-cured hams.

In reference to the non-protein nitrogen content, Type II hams showed less and Type IV more than the fresh hams. There was no significant difference between Types I, III, and the fresh hams and hardly any noticeable differences among the brine-cured hams in comparison with each other. The Type IV hams, however, did show a relatively greater non-protein nitrogen content.

The comparatively small amount of soluble nitrogen and non-protein nitrogen present in the brine-cured hams may have been due to the extraction of these nitrogen components by the brine solution during the curing and to some extent by the denaturation of the protein. The high non-protein nitrogen values observed in the Smithfield hams (Type IV) were largely due to enzymatic proteolysis which occurred during the long processing and storage periods.

The low soluble protein nitrogen content of the cured hams calculated as the difference between the soluble nitrogen and non-protein nitrogen, compared with the fresh hams, indicates the high degree of denaturation of the protein in the former. The Type IV hams showed an unexpectedly higher level of soluble protein nitrogen due possibly to some of the complex insoluble forms of protein which were broken down to the lower molecular weight soluble proteins. In addition, denaturing agents such as heat during smoking, concentration of salt used

TABLE 2

Significance of differences in chemical composition between various types of hams [b]

Types compared		Moisture	Protein	Fat	Sodium chloride	Soluble nitrogen	Non-protein nitrogen	Free fatty Acid	Peroxide value
Fresh	Tender	**	ns	**	**	**	ns	ns	ns
	Ready-To-Eat	**	ns	**	**	**	*	ns	ns
	Long Cure	**	ns	**	**	**	ns	ns	ns
	Smithfield	**	**	**	**	ns	**	**	**
Smithfield	Tender	**	**	**	**	**	**	**	**
	Ready-To-Eat	**	**	**	**	**	**	**	**
	Long Cure	**	**	**	**	**	**	**	**
Long Cure	Tender	ns	ns	ns	**	ns	ns	ns	*
	Ready-To-Eat	ns	ns	ns	**	*	ns	*	ns
Ready-To-Eat	Tender	ns	ns	ns	ns	**	*	ns	ns

[b] The sign ** indicates results significantly different at the 1% level; * results significantly different at the 5% level; "ns" results not significantly different.

in curing, and bacterial and native enzymes, may account for these variations.

Free fatty acids and peroxide values. There appeared to be no marked differences between the brine-cured and fresh hams with respect to either the free fatty acids or the peroxide values. There was a significant difference in free fatty acid content between Types II and III hams and likewise, in peroxide values between Types I and III hams. The Type IV hams had significantly higher values (Table 2) than the brine-cured and fresh hams, due to fat hydrolysis and oxidation respectively, which occurred during the lengthy smoking treatment of 30 to 40 days, and storage periods ranging from 7 to 20 months at room temperature.

The pH range of 5.92 to 6.17 showed little variation in relation to the types of curing. However, it is of interest to note that the Type IV hams had a lower pH value of 5.92, similar to that of the fresh hams, possibly attributable to the higher free fatty acid content.

As shown in Table 1, the ash content was proportional to the amount of chlorides present.

SUMMARY

A survey of the commercial methods of curing hams showed that there are four predominant types of curing, namely, tendered, ready-to-eat, long brine-cured, and the Smithfield process. Representative samples of these four types of curing were obtained for chemical analyses from large, intermediate, and small packing companies, and compared with fresh hams.

The various types of cured and fresh hams were analyzed for moisture, protein, fat, chlorides, soluble nitrogen, non-protein nitrogen, free fatty acids, peroxide values, and pH.

In general, the brine-cured hams were quite similar in composition. To a large extent, the Smithfield type showed highly significant differences in composition in comparison to the other types due to the more drastic curing and aging processes.

It is clearly shown that different curing procedures involving such factors as concentration of curing solution, dehydration resulting from high and lengthy smoking temperatures, long aging periods, and enzymatic action affect the chemical composition of these cured products.

Acknowledgment

The authors wish to acknowledge the assistance of Dr. H. R. Kraybill, Director, Department of Scientific Research, American Meat Institute, and Mr. C. B. Heinemann, President of the National Independent Meat Packers Association, as well as numerous members of these organizations, for their suggestions regarding typical commercial brine curing methods for hams; and the assistance of several producers of Smithfield hams located in Smithfield, Virginia, in describing methods of producing Smithfield hams.

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Rapid Versenate Methods for Determining Calcium in Solutions and Fruit and Vegetable Tissue for Quality Control[a,b]

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(Manuscript received June 12, 1952)

Rapid, simple colorimetric titration procedures employing the versenate technique for the determination of calcium in fruit and vegetable processing plant control analysis are described. Apples, tomatoes, and potatoes were the materials used in the investigation. Elimination of interfering substances is discussed.

There is a need in the food processing industry for a simple, rapid, yet relatively accurate method for calcium determination. This method should be applicable to in-plant operation for determining and maintaining the calcium content of fruit and vegetable firming baths. Also, since there are regulations *(3, 13)* governing the amount of calcium permissible in the finished product, a practical method is desirable for determining the calcium present in the tissue of the treated fruit or vegetable. This procedure would be particularly useful where the calcium is added directly to individual cans of the product.

The standard gravimetric and oxalate-potassium permanganate volumetric methods *(1)* require relatively expensive equipment and are time consuming. Williams and Bevenue *(15)* have suggested a modified soap method for determination of calcium in apple-firming baths. This method, however, may be improved from

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the standpoint of speed, accuracy, and simplicity. Simple volumetric procedures for determination of calcium based on the principles announced by Schwarzenbach, Biedermann, and Bangerter *(10)*, and Schwarzenbach and Gysling *(11)* employing disodium dihydrogen versenate (disodium dihydrogen ethylenediamine tetraacetate dihydrate) as the sequestering agent and ammonium purpurate (murexide) as the indicator have been described recently for water analysis *(2, 7, 9)*. This direct colorimetric versenate titration now is being adapted to other types of analysis. Wilson *(16)* has applied the procedure of Betz and Noll *(2)* satisfactorily to the estimation of calcium in wet ashed citrus leaf tissue solutions. Greenblatt and Hartman *(6)* reported excellent agreement of the versenate titration with the oxalate-permanganate method on biological fluids except in the case of jaundice and hemolytic serums. Ionic interference studies *(2, 9, 14)* indicated wide possible use of this procedure. Goetz, Loomis, and Diehl *(5)* showed the satisfactory stability of the standard versenate and calcium chloride solutions.

Because of its simplicity, accuracy, and apparent adaptability the versenate procedure was investigated to determine if it could be developed as a satisfactory plant control method for quickly estimating calcium in a, solutions used for firming apples or other fruit or vegetables, and b, in the tissue of the treated fruit or vegetable.

METHOD FOR FIRMING SOLUTIONS

Preliminary investigations during 1950 under experimental and commercial conditions indicated the feasibility of the direct titration procedure for determining calcium in calcium chloride apple-firming baths. Further studies on the method, including possible interferences not previously investigated that might be encountered, confirmed its suitability. The substantiating data are reported here.

Procedure. The procedure finally developed for plant control purposes has been described by Strachan *(12)*. The reagents and titration procedure are essentially those of Betz and Noll *(2)*.

Results. Results of single determinations on different experimental and commercial samples by the versenate procedure and the reference oxalate—permanganate method *(1)* are given in Table 1.

The data indicate good agreement between the versenate titration and the official permanganate procedure. With few exceptions the versenate values checked well within 1% of the reference method over the range 18 to 1131 p.p.m. of calcium. Some of the firming bath solutions (No. 6, 7, 11, 13) had had 4 to 6 lots of fruit passed through them increasing the soluble solids content of the bath solution to 6 to 10% due to leaching from the fruit tissue. An appreciable amount of suspended material which developed from some disintegration of fruit tissue was removed by filtration through No. 4 Whatman filter paper or glass wool prior to analysis. In addition to calcium chloride and fruit soluble solids, solution No. 9 contained 3% sodium chloride. A determination from sampling to completed titration required 2 to 3 minutes.

A few tests were made of applying the permanganate method directly to the firming solution without previous ashing. The values obtained were almost as accurate as by the versenate titration, but the procedure was not nearly as simple or rapid.

TABLE 1
Versenate determination of calcium in apple-firming solutions compared to the permanganate method

Conditions	Sample number	Versenate method	Permanganate method	Absolute difference	Relative error
		p.p.m.	*p.p.m.*		%
Experimental	1	189	184	+5	+2.7
	2	187	179	+8	+4.5
	3	1055	1050	+5	+0.5
	4	1027	1035	−8	−0.8
	5	913	910	+3	+0.3
	6	1129	1131	−2	−0.2
	7	1091	1089	+2	+0.2
	9	320	311	+9	+2.9
Factory	10	18	18	0	0
	11	238	240	−2	−0.8
	12	194	194	0	0
	13	246	243	+3	+1.2
	14	192	191	+1	+0.5
	15	252	251	+1	+0.4
	16	200	199	+1	+0.5
	17	268	267	+1	+0.4
	18	216	215	+1	+0.5
	19	240	239	+1	+0.4
	20	194	193	+1	+0.5

Limited studies were made of the versenate titration procedure for total hardness employing Eriochrome black T as the indicator *(2, 4, 8)* as a method for controlling the strength of calcium-firming solutions. While this procedure could be used, it lacked the directness and accuracy of the murexide calcium titration.

Interferences. Fruit and vegetable firming solutions may contain relatively high concentrations of leached soluble solids, from plant tissues, sodium chloride, sulphite, sugar syrup, or starch. The possible interferences in the determination by these substances were investigated. Leached soluble solids in concentrations that might be attained apparently did not affect the accuracy of the versenate method for calcium nor did 3% sodium chloride solutions (Table 1, sample No. 9). Suspended organic material of disintegrated tissue will result in a sliding end point unless removed by filtration. In preparing products for freezing, SO_2 or potassium metabisulphite may be added to the calcium firming solution. Concentrations of SO_2 up to 6,000 p.p.m. as SO_2 and possibly higher, did not affect the precision or accuracy of the method. Sugar syrups in strengths that may be encountered in the diluted sample being titrated, do not affect the accuracy of the versenate titration. However, in extreme cases of high sugar concentration, as for instance with 50% sugar syrup in the unknown being titrated, the results for calcium will be greater than the true value by approximately 20 p.p.m. when the calcium level is around 400 p.p.m. Concentrations of citric acid up to 1% in the titration sample were found to have no effect on the accuracy or clearness of the end point. Starch also does not interfere.

While calcium chloride is the salt most generally used in firming solutions, other calcium compounds which may be employed as firming agents are calcium sulphate, calcium citrate, calcium lactate, calcium malate, calcium gluconate, and monocalcium phosphate. In addition to calcium chloride, satisfactory results were obtained with the versenate technique for calcium sulphate and calcium citrate. The method could not be

used for monocalcium phosphate. Its suitability for the other calcium salts is not known but from a chemical point of view the method should be satisfactory.

METHODS FOR FRUIT AND VEGETABLE TISSUE

Since a method primarily adapted to processing plant control requiring a minimum of equipment, time, and skill was desired for determining calcium in tissue, it was assumed that an accuracy within 10% would be satisfactory. However, a more accurate procedure approaching the gravimetric or oxalate-permanganate methods but requiring less time also would be very desirable.

There appeared to be two approaches to obtaining such a method. One may apply the versenate titration to solutions of the ashed material as successfully done by Wilson *(16)* on citrus leaves; or apply it to properly clarified and buffered solutions representing the acid extracts of macerated tissues. The former procedure seemed to offer the possibilities of greater accuracy; the latter, greater simplicity and rapidity.

The materials used in these investigations were mostly experimentally prepared canned samples of apples, tomatoes, and potatoes which had been subjected to varying degrees of calcium chloride firming treatment. Two of the potato samples were commercial packs with added calcium chloride. The reference method employed was the volumetric oxalate-permanganate procedure *(1)*.

Methods for Ashed Tissue

Procedure. The 25-g. samples were ashed *(1)* in porcelain dishes. The ash was dissolved in 50 ml. of hydrochloric acid (1 + 4) by warming on a water bath, then transferred to a 250 ml. volumetric flask, cooled, and neutralized with 40% sodium hydroxide to approximately pH 7. Distilled water was added to bring the total volume to 250 ml. at 20° C. A 50-ml. aliquot was adjusted to pH 12 with sodium hydroxide and titrated with standard versenate as for firming solutions *(12)* using a 5 ml. microburette. A preliminary titration should be made to establish the approximate end point. The end point adopted was that at which no further color change took place. The blank determination titer should not exceed 0.1 ml.

Results and discussion. The results for apples were in fairly good agreement with the permanganate method but the values obtained for tomatoes and potatoes were low, being only one-third to one-half those of the reference method (Table 2). The versenate values for apples checked within 5% of those obtained by the permanganate procedure but generally were slightly lower. This tendency to a slightly lower value probably was due to the faint precipitate formed on neutralizing. A moderate to heavy flocculant precipitate occurred on neutralizing the solutions from tomato and potato tissues. Possibly their much greater phosphorous content resulted in precipitation of some of the calcium at the strongly alkaline pH required for the versenate procedure. Tomatoes and potatoes also have a much greater ash content, especially in relation to their calcium content, as compared to apples. This may be a contributing factor to the unreliable results obtained with these two products. The end point with the tomato and potato samples was too indefinite to be of value but was satisfactory for apples.

TABLE 2

Versenate determination of calcium on ashed samples of canned apples, tomatoes, and potatoes compared to the permanganate method

Product	Versenate method	Permanganate method	Absolute difference	Relative error
	p.p.m.	*p.p.m.*		%
Apples	38	40	− 2	− 5.0
	50	52	− 2	− 3.8
	62	60	+ 2	+ 3.3
	83	87	− 4	− 4.6
	111	113	− 2	− 1.8
	122	120	+ 2	+ 1.7
	156	157	− 1	− 0.6
	216	210	0	0
	296	310	− 14	− 4.5
	315	321	− 6	− 1.9
	532	555	− 23	− 4.1
	1053	1090	− 37	− 3.4
Tomatoes	31	49	− 18	− 36.7
	41	55	− 14	− 25.4
	158	163	− 5	− 3.1
	265	289	− 24	− 8.3
Potatoes	41	55	− 14	− 25.4
	31	69	− 38	− 55.0
	53	86	− 33	− 38.4
	117	227	− 110	− 48.4

Method for Acid Extract of Tissue

Procedure for apples and tomatoes. The freshly treated or canned sample was thoroughly mixed in a Waring blendor. Then 100 g. of the macerated sample was blended with 200 ml. of hydrochloric acid (1 + 4) for 5 minutes, made up to 500 g. with distilled water and filtered through No. 41 H Whatman filter paper. Approximately 2.5 g. (1 tablespoon) of reagent grade Darco G 60[c] (calcium and phosphorus free) activated charcoal was added to 200 ml. of the filtrate and the contents allowed to stand for 30 minutes with intermittent shaking. After filtering through No. 4 Whatman filter paper, 100 ml. of the decolorized filtrate was made up to a volume of 200 ml. with distilled water. For titration 25-ml. aliquots were transferred to 250-ml. beakers or evaporating dishes, diluted with distilled water to about 50 ml., and adjusted to pH 12 with 20% sodium hydroxide (approximately 3 ml.). To control interfering ions, particularly copper contamination from metal parts in the Waring blendor during the acid extraction, it was found necessary to add 2 drops of 10% potassium or sodium cyanide or 2 crystals of sodium sulphite prior to the versenate titration. Murexide indicator was added and titration was completed with standard versenate solution (1 ml. equals 0.4 mg. of calcium). The blank determination titer should not exceed 0.2 ml.

The tissue solutions contained coloring matter which obscured the titration end point. Therefore the suitability of a number of decolorizing agents and procedures were investigated including amyl alcohol, neutral lead acetate, alumina cream, and several activated carbons. Of these, only Darco G 60 reagent grade activated carbon and alumina cream yielded accurate results. Darco G 60 gave a small consistent blank and was not critical as to the quantity used within ± 0.5 g. of the amount indicated for decolorizing. Alumina cream, prepared from a saturated solution of alum and ammonium hydroxide could be used as a substitute for the Darco carbon but the latter was preferred.

Procedure for potatoes. In the case of potatoes, the high concentration of starch naturally present interfered with filtration. Therefore, instead of using the 5 minute acid blend to extract the calcium as previously described, the acidified sample was gently boiled for 15 minutes. This treatment hydrolized the starch permitting rapid filtration. The remainder of the procedure was unchanged.

The end point obtained on tissue solutions was not as sharp as that on firming solutions but with a little practice it can be judged with a reasonable degree of precision. Good lighting

[c] Atlas Powder Company, New York.

and also a white base titration stand if a beaker is used are essential to determine accurately the color change.

Results and discussions. The procedure as outlined required 1.5 hours as opposed to 2-3 days for the permanganate method. It proved suitable for rapid anaylsis of apples, tomatoes, and potatoes (Table 3 and 4). Of

TABLE 3
Versenate control method for determination of calcium in canned apples compared to the permanganate method

Versenate method	Permanganate method	Absolute difference	Relative error
p.p.m.	*p.p.m.*		%
22	15	+ 7	+ 46.7
39	40	− 1	− 2.5
57	50	+ 7	+ 14.0
59	52	+ 7	+ 13.5
58	54	+ 4	+ 7.4
57	59	+ 2	+ 3.5
66	60	+ 6	+ 10.0
60	61	− 1	− 1.6
67	65	+ 2	+ 3.1
77	74	+ 3	+ 4.1
82	75	+ 7	+ 9.3
96	87	+ 9	+ 10.3
115	113	+ 2	+ 1.7
132	120	+ 12	+ 10.0
135	126	+ 9	+ 7.1
129	138	− 9	− 6.5
139	144	− 5	− 3.5
148	146	+ 2	+ 1.4
163	157	+ 6	+ 5.7
175	182	− 7	− 3.8
218	216	+ 2	+ 0.9
306	310	− 4	− 1.3
312	321	− 9	− 2.8
374	380	− 6	− 1.6
559	555	+ 4	+ 0.7
1001	1090	− 89	+ 8.2
1062	1091	− 29	− 2.7

TABLE 4
Versenate control method for determination of calcium in canned tomatoes and potatoes compared to the permanganate method

Product	Versenate method	Permanganate method	Absolute difference	Relative error
	p.p.m.	*p.p.m.*		%
Tomatoes	50	49	+ 1	+ 2.0
	53	55	− 2	− 3.6
	60	58	+ 2	+ 3.5
	95	93	+ 2	+ 2.2
	160	159	+ 1	+ 0.7
	160	163	− 3	− 1.8
	194	203	− 11	− 5.4
	208	207	+ 1	+ 0.5
	285	282	+ 3	+ 1.1
	297	289	+ 8	+ 2.8
Potatoes	48	47	+ 1	+ 2.1
	55	55	0	0
	64	69	− 5	− 7.2
	79	76	+ 3	+ 3.9
	89	86	+ 3	+ 3.5
	235	227	+ 8	+ 3.5

43 samples analyzed, all but 4 gave values checking within 10% of the reference method. In 29 of the samples the error was less than 5%. The few samples where the relative error exceeded 10% occurred in the low concentrations of less than 100 p.p.m. of calcium.

The Food and Drug Regulations of Canada *(13)* and the United States *(3)* permit a maximum calcium content in canned tomatoes of 260 p.p.m. For potatoes the United States regulations allow a maximum of 510 p.p.m. of calcium *(3)*. Other products to which calcium might be allowed to be added in the future likely would have similar tolerances. In the range of 100 to 1000 p.p.m. of calcium the rapid versenate procedure described for tissue extracts may be expected, after a little experience, to give results at least within 10% of the permanganate value. The method could be very useful in keeping the product within the prescribed tolerance.

SUMMARY

Rapid, simple titration methods well adapted to determining calcium in fruit and vegetable processing plant work have been developed employing disodium dihydrogen versenate as the sequestering reagent and murexide as the indicator. Apples, tomatoes, and potatoes were the materials used in the investigation.

For determining the calcium content of firming baths a procedure is described which may be performed in 2 to 3 minutes with an accuracy of around 1%.

For estimating calcium in fruit tissues the versenate titration technique was applied to decolorized acid extracts. While experience was required in determining the end point, an accuracy within 10% and frequently within 5% of the reference oxalate-permanganate method was obtained. A determination required only 1½ hours.

The versenate procedure applied to solutions of ashed material proved unsatisfactory for tomatoes and potatoes, but values for apples showed less than 5% error.

Applicability of the methods are discussed. As developed, the technique is sufficiently simple for use by non-technical operators. Inexpensive standard laboratory equipment and readily available reagents are used.

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The Defrosting Method and Palatability of Poultry[a]

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The methods for defrosting frozen poultry are of interest because of the comparative time required for defrosting and because of their possible effect on the acceptability of the cooked product. The results of defrosting during cooking, in the refrigerator, at room temperature, and in water upon the cooking time, loss in weight during cooking, and palatability of the cooked product are herein reported.

The results of many studies have been published concerning the effects of evisceration, freezing methods, the type of packaging, the storage temperature, and the changes occurring during the storage of poultry. Little information is obtainable concerning the effects of defrosting methods upon the palatability of frozen poultry. This study was undertaken to investigate the desirability of four defrosting methods. The criteria of desirability were subjective palatability scores and objective tenderness ratings.

PROCEDURE

Considerable variation in quality from one bird to another has been found in this laboratory. The right and left sides of the same bird, however, have the same heritage, the same feeding history, the same composition (particularly fat), and the muscle proteins behave similarly to heat. When paired treatments are being used in a study, considerable variability can be eliminated by using the right and left sides of the same bird. Paired halves were used in these studies. Each roaster was divided into halves while still frozen by sawing through the back and keel bones with a band saw. When the keel bone is split, the skin is left attached to the keel bone and the breast muscles are better protected from drying during cooking.

The roasters were divided into three groups:

I. One half of a bird was defrosted slowly in a refrigerator held at 41° F. (5° C.) and the other half during cooking in an oven at 302° F. (150° C.).

II. One half of a bird was defrosted in the refrigerator and the other half at room temperature, 81-89° F. (27-31° C.).

III. One half of a bird was defrosted in the refrigerator and the other half in water.

Preparation of poultry. For groups I and II, grade A roasters weighing 4-4.9 pounds were obtained in Omaha in November. They were New York dressed and cooled to about 41° F. (4.5° C.). Each bird was individually wrapped in cellophane and enclosed in stockinet. The roasters were then packed 6 to a carton, frzoen, and stored at —5° F. (—20.6° C.). In March the pack was prepared for shipment to Ames. This consisted of thawing, eviscerating, and then refreezing at —40° F. (—40° C.) in a blast tunnel. The roasters were packed in dry ice for shipment to Ames. At Ames the shipment was stored in the only space available 10° F. (—12.2° C.) for about 3 months. During the period of the cooking tests, which lasted 6 weeks, half of the roasters were stored at 0° F. (—17.8° C.), the other half at —10° F. (—23.3° C.).

The roasters for group III were selected and packaged in October in Omaha. They weighed 3.5-4.5 pounds. They were eviscerated as soon as the picking operation was completed. The birds were laid on a piece of wax paper 7 x 10 inches. They were than packed in waxed cartons and frozen. The pack was then shipped to Ames and stored at —10° F. (—23.3° C.).

Defrosting and cooking. The two halves of a given roaster were always cooked and scored the same day. Hence, the two half-roasters were not always removed from frozen storage at the same time. Each half-roaster defrosted in the refrigerator or room was placed on a tray, and the tray enclosed in a pliofilm bag. The half roasters defrosted during cooking and those defrosted in water were taken from storage the day they were cooked. The amount of water for defrosting a half-roaster was 3.5 liters. The initial temperature of the water was 77° F. (25° C.). The half-roaster and container were then left at room temperature.

The defrosting and cooking treatment was randomized, so that half of the half-roasters defrosted by a given method were from the right, the other half from the left side of the bird.

Each half-roaster was placed skin side upon a rack in a pan and cooked in an oven heated to 302° F. (150° C.). Progression of cooking was followed by placing the bulb of a short stem thermometer in the thick part of the thigh muscles. Each half-roaster was removed from the oven when the interior of the thigh muscle reached 185° F. (85° C.).

Palatability tests. Four half-roasters were cooked at a time, each in a separate oven. All samples were coded. Cross sections of the thigh and the two breast muscles were scored for palatability. The two breast muscles were scored separately for group III. All scoring was done by a trained panel. Each scorer was given, as nearly as possible, the same anatomical section of each muscle(s) for scoring. The samples were scored for aroma, flavor, tenderness, and juiciness. A 1-10 point scale was used for each quality factor, with 10 high.

The posterior portion of the pectoralis major muscle which remained after the organoleptic test samples had been removed was used for determining the shear force values. Seven shears were obtained on this portion of the muscle. A modified Warner-Bratzler shearing apparatus was used for the tests.

RESULTS

Weight lost during defrosting. The weight lost during defrosting was affected by the defrosting time as shown in Table 1. The half-roasters in the refrigerator for 48 hours lost twice as much weight as those in the

TABLE 1

The defrosting method, number of half-roasters used, the average weight of half-roasters, defrosting time, thigh temperature at start of cooking, and cooking time

Group	Defrosting method	No. of half-roasters	Weight of half-roasters	Defrosting time	Thigh temperature at start of cooking	Cooking time
			g.	*hours*	*° C.*	*minutes*
I	Refrigerator	30	959[d]	42.1	2.5	95.7
	Oven	30	915[d]	.....	—13.0	137.3
II	Refrigerator	30	946[d]	47.5	4.8	103.7
	Room	30	917[d]	6.3	4.7	100.0
III	Refrigerator	40	646	23.2	1.0	79.0
	Water	40	645	0.58	1.6	76.0

[d] Includes portion of giblets in half-roaster when sawed into halves.

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refrigerator for 24 hours. The half-roasters defrosted at room temperature lost 1.54% weight during defrosting and an additional 1.31% during the holding period in the refrigerator. Half-roasters thawed in water gained weight.

The ice in muscle thaws at about 28° F. (2° C.). The average thigh temperature at the start of cooking (Table 1) indicated that the ice in the muscles had melted; however, the muscles of a majority of the half-roasters did not reach refrigerator temperature, 41° F. (5° C.).

Cooking time. The half-roasters which were defrosted during cooking required about one and one-half times as long for cooking (137.3 minutes) as the corresponding halves which were thawed in the refrigerator (95.7 minutes). The cooking time was influenced by the size of the roasters, the larger half-birds requiring a longer time than the smaller ones.

Cooking weight losses. As shown in Table 2, the weight lost during cooking of the half-roasters of group I, defrosted in the refrigerator, was smaller than for the corresponding half-roasters thawed in the oven.

TABLE 2

Method of defrosting and average defrosting weight losses, cooking weight losses, and total loss (defrosting, holding, and cooking)

Group	Defrosting method	Defrosting weight loss	Cooking weight losses		Total weight loss
			Volatile	Total	
		%	%	%	%
I	Refrigerator	2.05	17.2	20.2	22.2
	Oven	.....	20.0	23.7	23.7
II	Refrigerator	2.25	18.9	21.9	24.8
	Room	1.54	18.8	21.5	23.8
III	Refrigerator	1.10	18.2	20.0	20.4
	Water	0.98*	18.0	20.1	19.1

* Gain in weight.

Part of this loss may have been caused by the much longer cooking time needed for the frozen samples. The total weight losses (which included the weight lost during defrosting, that lost while holding in the refrigerator, and that lost during cooking) of the half-roasters thawed in the oven and refrigerator were 23.7 and 22.2%, respectively. There was very little difference in the weight losses during cooking between the halves of half-roasters defrosted in the refrigerator and those defrosted at room temperature for group II or for halves defrosted in the refrigerator and water for group III. The slight differences in the total weight losses was largely because of differences in weight lost during defrosting.

TABLE 3

Average palatability scores of half-roasters defrosted in the refrigerator, the oven during cooking, at room temperature, and in water

Group	Muscle	Defrosting method	Scores			
			Aroma	Flavor	Tenderness	Juiciness
I	Breast	Refrigerator	7.8	7.3	8.4	5.8
		Oven	7.8	7.4	8.4	5.6
	Thigh	Refrigerator	6.8	5.9	7.5	6.5
		Oven	6.6	6.0	7.2	6.1
II	Breast	Refrigerator	7.6	7.1	8.9	4.9
		Room	7.7	7.1	8.9	4.7
	Thigh	Refrigerator	6.8	5.7	7.9	5.6
		Room	6.9	6.0	8.0	5.5
III	Breast (large)	Refrigerator	7.9	6.6	7.5	6.3
		Water	7.9	6.3	7.7	6.1
	Breast (small)	Refrigerator	7.9	6.2	8.7	6.2
		Water	7.7	6.2	8.7	5.9
	Thigh	Refrigerator	7.8	6.5	7.4	6.8
		Water	7.9	6.5	7.3	6.6

Appearance. Some desiccated areas were evident on the skin of the roasters when they were removed from storage for cooking. There were no observable differences in the appearance of the half-roasters after cooking that could be attributed to the method of defrosting.

Palatability. There were no significant differences between the aroma, flavor, tenderness, and juiciness scores for the two treatments of the half-roasters in each group. These results are in agreement with those of Beery, Prudent, and Wilson *(1)*. In general, the differences in the averages between the two treatments for the paired half-roasters were less marked for each of the three groups of the study than differences from roaster to roaster.

The differences in the average shear force values between the two treatments for each group of paired half-roasters was negligible. The shear force values followed closely the general trend of the tenderness scores.

SUMMARY

Four comparisons of methods of thawing (in oven, refrigerator, room, and water) poultry were made. The effects on cooking time, weight, and palatability were evaluated, using half carcasses in each comparison. Defrosting and cooking times and weight changes varied significantly between methods. No significant differences in palatability between treatments were noted.

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Fungal Enzymes in Bread Baking

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The sources, standardization, concentration, and types of fungal enzymes applicable to bread making are reviewed, together with the effects of these enzymes on the finished product.

Malted wheat and malted barley have been used traditionally in the milling and baking industry as enzyme supplements to modify wheat starch and to increase fermentable sugars. During the past years enzymes derived from microorganisms have been developed and tested extensively in bread baking. The findings of fact of the Standards of Identity for Bread and Rolls *(4)* state: "Recently it has been found that enzymes having somewhat similar action [to malt] on the starch of flour can be obtained from media in which certain molds, particularly, *Aspergillus oryzae*, are grown. Purified preparations containing enzymes from *A. oryzae* are suitable for use in bread and rolls." Therefore, the standards permit the addition of "malt sirup, dried malt sirup, malted barley flour, malted wheat flour, each of which is diastatically active; harmless preparations from *A. oryzae*, or any combination of two or more of these" as optional ingredients. The addition of fungal enzymes to flour is not permitted under the present Standards of Identity for flour but it is hoped that the flour standards can be revised to permit the addition of such enzymes at the mill.

Fungal enzymes are produced commercially by growing the microorganism on a suitable medium of edible materials, usually wheat, wheat bran, or corn. After extraction of the medium and precipitation of the active enzymes from the extract a relatively pure preparation is obtained *(13, 18, 24)*. The purified enzyme powders vary somewhat in enzymatic activity from batch to batch and require the addition of a suitable, edible ingredient for purposes of standardization. Enzymes which are used in the food industries are generally standardized with sugars, such as dextrose, or with cornstarch, flour, or salt *(18)*.

Fungal enzyme concentrates are frequently diluted further than would be required merely for purposes of standardization. Concentrations of fungal enzymes which are useful in bread baking are frequently of the order of 0.0025 to 0.005% (based on the weight of flour) and sensitive balances which are generally not available in the average bakery would be required to weigh the enzyme concentrate necessary for a 400 to 600 pound dough. Therefore, it is customary to dilute fungal enzyme concentrates with sugars, starch, or flour to a point corresponding to the addition of 4 ounces of the diluted material to 100 pounds of flour which can readily be handled in the average bake shop. The use of other baking ingredients, such as yeast foods, as diluents or functional ingredients in combination with enzymes has been suggested *(25)* and several preparations combining fungal enzymes with yeast food or with cereal malt are commercially available.

[a] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Michigan, June 11, 1952.

STANDARDIZATION

The baking test is the final criterion as to the usefulness of an enzyme preparation. Nevertheless, it is desirable to standardize enzyme preparations on the basis of a quantitative test which correlates reasonably well with the final baking result. The determination of alpha amylase activity, of the "maltose value," of gassing power, and of the amylograph value (a viscosity method) are now practiced in the milling and baking industry *(1, 21)*. Of these methods the amylograph is inapplicable to fungal enzymes because they are inactivated by the temperature required to gelatinize the raw starch or flour with which they are heated. The determination of alpha amylase activity according to Sandstedt, Kneen, and Blish *(19)* correlates well with the results of baking tests. It can be measured independent of the action of other enzymes which are present in flour and it is therefore the best method for standardizing fungal diastatic preparations. Determination of the "maltose value" and of gassing power may be suitable if the limitations of the method, that is, the dependence on a variable substrate, are kept in mind.

Diastatic fungal enzyme preparations are generally available in many stages of dilution varying from 50 to 500 SKB (alpha amylase units) for so-called dilute materials up to 20,000 units per g. for the concentrates. In comparing a fungal enzyme concentrate of 5,000 SKB units per g. with an average malted wheat flour of about 40 to 45 SKB units per g. it will be found that the fungal preparation is about 100 times as active in a baking test, that it is from 50 to 100 times as active in gassing power test and that it is only about 15 times as active in the "maltose test."

Determination of proteolytic activity of flour or cereal enzymes is not generally practiced in the baking industry. The introduction of fungal enzymes in bread baking has made it necessary to find suitable methods and use them in standardizing the enzyme preparations, at least on the part of the supplier. At present a variety of substrates are used since wheat gluten of sufficient purity and uniformity is not commercially available. Of the methods suggested Miller's modification of the Ayre and Anderson method in which hemoglobin is used as the substrate is one of the more useful *(14)*.

APPLICATION OF FUNGAL ENZYMES IN BREAD BAKING

Twenty-five to thirty-five years ago the use of fungal enzymes from *A. oryzae* in bread baking was described in several patents *(11, 12, 23)*. However, it is unlikely that this early work resulted in any commercial use of such enzymes. The most extensive and systematic work with fungal enzymes has been carried out by Shellenberger, Johnson, and Miller working at the Dept. of Milling Industry at Kansas State College *(3, 6, 7, 8, 9, 10, 15, 16, 17)*. Most of their work with unmalted

wheat flour was carried out on an experimental laboratory or pilot plant scale.

In comparing fungal enzyme concentrates with malted wheat flour on an equivalent alpha amylase basis they found that the fungal enzyme was entirely satisfactory for bread baking based on their evaluation of grain, texture, crust and crumb color, and loaf volume. They separated the fungal diastase and protease and applied them separately in varying concentrations. In contrast to malt a large excess of fungal alpha amylase could be tolerated (without leading to the development of sticky bread crumb) but they found no advantage in using more alpha amylase than 5,000 to 10,000 SKB units per 100 pounds of flour (corresponding to about 0.25% of wheat malt). Increasing concentrations of fungal protease led to a rapid and continuing decrease in dough viscosity and the concentration of protease must be well controlled in bread baking. Their results were applied to pilot plant tests with 5 hard red spring and 5 hard red winter wheat flours. In each case a slightly different ratio of diastatic and proteolytic fungal enzymes gave optimum results which were frequently superior to those obtained with malted wheat flour.

Harrell, Lincoln, and Gunderson *(5)* have worked with a fungal alpha amylase preparation which was very low in proteolytic activity. They obtained bread that possessed better color, texture, grain, and volume than could be obtained with malted wheat or malted barley flour at equivalent Blish-Sandstedt "maltose values." It should be noted that they have worked with fungal diastase concentrations from 5 to 7 times higher than those employed by Shellenberger and co-workers who compared fungal enzymes and wheat malt on the basis of equivalent alpha amylase activity. They believe that the addition of fungal protease is generally harmful on normal flours and that its use should be restricted to certain flours which give very bucky doughs.

The results of the first large scale commercial use of fungal enzymes were reported by Skovholt *(22)*. Results obtained with almost 200 doughs using 4 different fungal enzyme preparations and malt extract were averaged. There were slightly higher scores for the crumb color, external characteristics, and loaf volume for the fungal enzyme although the averages included some rather unfavorable results with fungal enzymes resulting from experimental trials to find optimum conditions. Shop studies showed that there was a somewhat greater rise in sponge temperature and usually a quicker "pick-up" in the mixer when fungal enzymes were used instead of malt extract. The effect on absorption was minor and in some cases there was an increased tendency to stickiness probably because of the presence of excess protease.

Coles *(2)* investigated the influence of a fungal protease on mixing time and bread quality. Working with a fungal enzyme which had been diluted to a protease concentration approximately 10 times that of a malted wheat flour he could reduce his mixing time to 84%, 72%, and 55% of normal by adding $\frac{1}{16}$th, $\frac{1}{8}$th, or $\frac{1}{4}$th percent of the fungal enzyme, respectively. Higher concentrations of the fungal enzyme reduced the mixing time still further but resulted in stickiness of the dough and produced a course, open grain and a lower loaf volume. For best results he "undermixed" doughs containing the fungal protease so that they were less extensible than control batches when they left the mixer but showed better extensibility when they reached the molder.

There has now been a good deal of commercial experience with the use of fungal enzymes in bread baking. It should be remembered that reports from commercial bakeries are based on the use of fungal enzymes which usually contain a mixture of protease and diastase and that this supplementation is superimposed upon the malt which is generally added at the mill. There is some indication that this combined use of fungal enzymes with malt has advantages although optimum levels of both materials for such combined use have not been established. The principal manufacturing advantages which have been reported from commercial users are a reduction in mixing time and better handling properties of the dough (better extensibility). Use of higher concentrations of fungal amylase gives drier doughs while higher concentrations of protease usually produce some stickiness.

Final bread quality is also favorably influenced by the commercial use of fungal enzymes. Improvement of grain and texture and external charactersitics and loaf volume have been reported. These improvements have been achieved after fairly extensive experimentation by individual bakeries and by the use of fungal enzymes relatively high in proteolytic activity in some cases and high in amylase activity in others. Improvement in grain, that is, a finer cell structure, seems to give the appearance of a whiter color which has been observed by several commercial users.

Similar to malt, fungal enzymes have a moderate effect in producing a softer loaf of bread. It is likely that the possibility of using much larger amounts of fungal amylase will make them more useful in this respect. The effect of fungal enzymes on bread softness is not as pronounced as with the best polyoxyethylene compounds. However, in some cases it has permitted a lowering of the concentration of emulsifiers in the formula. Recently, the use of a fairly heat stable diastase from bacterial sources has been suggested to retard bread staling *(20)*. A discussion of this application of a bacterial diastase is outside the scope of this review.

OUTLOOK

From a technical point of view it seems most desirable to add fungal diastase to the flour at the mill with or without the addition of malted flours. It is likely that the use of fungal enzymes in this manner will depend on their economy in comparison with malted wheat or malted barley flour. The use of appreciably higher concentrations of fungal diastase as suggested by Harrel may well be left to the judgment of the individual baker. For proteolytic supplementation a minimum amount of protease should perhaps be added by the milling industry directly to the flour, particularly for wheat varieties requiring very long mixing time or producing bucky doughs. In general, however, the use of a fungal protease should be made by the baker since proper dough extensibility and bread quality reacts sensitively to slight variations in protease depending upon differences in bake shop practices.

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Effect of Freezing and Thawing on the Growth Rate of Bacteria in Ground Meat[a]

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(Manuscript received May 31, 1952)

A comparison was made of the growth of psychrophilic bacteria and four well known mesophilic bacteria in fresh and frozen and thawed ground meat. Growth curves were drawn and generation times calculated to contrast the extent of lag and rates of growth in the frozen and unfrozen substrates. No indication was found in this limited study that frozen meat becomes more perishable after thawing than fresh meat.

An earlier report by this author *(8)* pointed out that when counts are made at 68° F. (20° C.) there is an actual increase in the number of bacteria recovered from frozen meat stored at 0° F. (—18° C.) over a 12-week period. The significance of this finding in terms of quality of the product depends entirely on the rate at which the organisms present can multiply in meat during the period between thawing and cooking. A very widespread popular belief holds that the rate of microbial growth is much faster after freezing and thawing than in the unfrozen material and consumers are frequently warned to use frozen foods promptly after thawing. These warnings often imply that there may be a serious public health hazard in holding thawed frozen meat for any length of time and that refreezing thawed meat is particularly dangerous. In view of the fact that psychrophilic bacteria are not likely to suffer any decrease in numbers during freezer storage and that they might be expected to cause rapid spoilage in thawed meat held at refrigerator temperatures, an investigation of their growth rate, and the growth rate of other microorganisms in thawed frozen meat seemed to be indicated.

[a] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Mich., June 9, 1952.

Although there are few studies dealing with growth rates of microorganisms in meat, the general problem of bacterial growth has occupied the attention of investigators for many years. Rahn *(6)* and Monod *(4)* have reviewed many of the principles discovered, giving special attention to the dynamics of growth and its mathematical treatment. Barber *(1)* in 1908 studied the generation time of *Escherichia coli* in single cell cultures at various temperatures from 53.6° to 116.6° F. (12° to 47° C.) and found growth to be extremely slow at 53.6° F. with a generation time of over 200 minutes. Penfold *(5)* in 1914 observed that although brief chilling of cultures in the logarithmic phase did not interrupt growth, chilling for longer periods (2 days) returned the organisms to a lag phase. More recently Hegarty and Weeks *(3)* have shown that cells of *E. coli* are sensitive to cold shock throughout the whole period of logarithmic growth. S. E. Hartsell *(2)* has studied what he very aptly terms "growth initiation" of bacteria in thawed frozen eggs but his work differs from the present report in that Hartsell held his thawed eggs at 98.6° F. (37° C.) and studied only organisms for which that was an optimum temperature. He noted that *E. coli* was apparently stimulated by being held in freezer storage and showed a greatly shortened lag phase after freezing and thawing.

EXPERIMENTAL METHODS

The meat used in these studies was prepared from the lean tissue of pork hams, or beef loins, by careful separation in the bacteriological laboratory so as to avoid contamination in so far as possible. The meat was cut into one-inch cubes, placed in a large evaporating dish, and inoculated by pouring over it an 18-hour broth culture of the organism being investigated. The

cubed meat was thoroughly mixed with a spatula to distribute the inoculum and was then ground twice through a ¼-inch plate and once through a ⅛-inch plate. Then 11 to 12 g. portions of the ground meat were stuffed into sterile plugged test tubes by using a calking gun in a manner similar to that employed by Stumbo, Gross, and Vinton *(7)* who filled thermal death time tubes with a grease gun. Immediately after filling, alternate tubes were placed in a freezer at —9.4° F. (—23° C.) and in a refrigerator at 44.6° F. (7° C.). Following the freezing period the tubes from the freezer were thawed for 30 minutes in a cold water bath and then placed in the 7° C. refrigerator.

Bacteriological sampling was accomplished by removing the tubes from the refrigerator at appropriate intervals and transferring 10 g. of ground meat from the test tube to a tared sterile Waring blendor jar, adding 90 ml. of sterile physiological saline, and blending for 30 seconds. Dilutions were then made in the conventional manner and plates poured with veal infusion agar (Difco veal infusion medium with 1.5% agar added). Plates were incubated at either 68° F. (20° C.) or 98.6° F. (37° C.) depending on the organisms being studied. Two test tubes were always sampled simultaneously to represent each time interval and duplicate plates were poured from each dilution. Thus counts were obtained and averaged from four plates at any one dilution.

With the exception of four experiments with mesophilic organisms, all the meat was inoculated with one of the group of psychrophilic organisms previously described by the author *(8)*. The particular organism used was one of the 14 strains described in Table 2 of the reference cited *(8)* and is similar to members of the *Pseudomonas* genus but is urease positive. The four mesophilic organisms used were selected for their sanitary significance or importance in food intoxication. Those used were: a strain of *Salmonella anatun* isolated from human sources,[b], *Salmonella choleraesius* isolated from a hog cholera source, an organism likely to be found as a contaminant in pork; a strain of *Escherichia coli* isolated in our laboratory from fresh pork trimmings; and a food poisoning staphylococcus, *Micrococcus pyogenes* var. *aureus*, number 9664 of the American Type Culture Collection. All plates used for counting these organisms were incubated at 37° C. while those for the psychrophilic organisms were incubated at 20° C. in accordance with our standard practice.

RESULTS AND DISCUSSION

The three curves in the lower portion of Figure 1 show the growth in three subdivisions of a lot of frozen and thawed meat during refrigerator storage at 44.6° F.

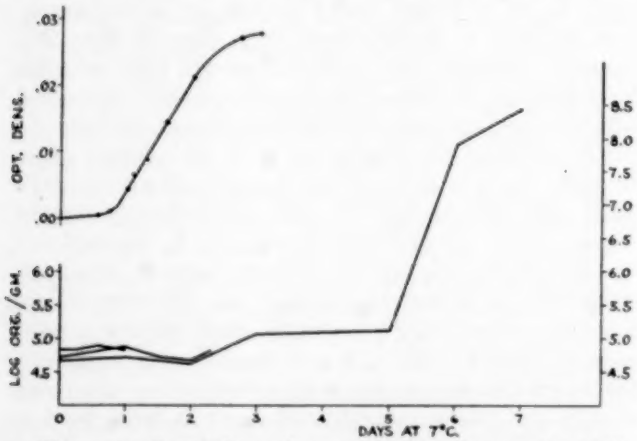

Figure 1. Comparison of growth rates of a psychrophilic bacterium in dextrose broth and thawed frozen meat at 44.6° F. (7° C.).

(7° C.). The three groups were removed from the freezer after 15, 29, and 34 days and appear on the graph in that order from top to bottom. The upper curve on Figure 1 is a growth curve of the same psychrophilic organism in frozen and thawed dextrose broth. The broth was inoculated with 1 drop of an 18-hour culture, held for 15 days in the same freezer used for the meat, thawed in cold water and held subsequently at 7° C. except that it was removed periodically to make optical density readings in an Evelyn photoelectric colorimeter. Counts for the three groups of meat resulted from the fact that the expected growth did not occur before all the tubes of the first group had been sampled and it was necessary to remove a second and finally a third group from the freezer. The curve for growth in frozen and thawed dextrose broth shows that a lag period of 18 hours was induced by freezing and that logarithmic growth began at about 20 hours. In meat, however, the lag phase persisted for at least 48 hours, asuming the small fluctuations during the first two days to be due to experimental error. There was evidently some acceleration in growth during the period between 2 and 5 days but the increase in count was not great. After 5 days the organisms entered the logarithmic phase. Since, from a practical viewpoint, we are concerned with meat preservation it is chiefly the duration of the lag phase of the growth curve that interests us.

Figure 2 summarizes the results of two experiments in which the growth of the psychrophilic bacterium in ground pork at 44.6° F. (7° C.) that had been frozen

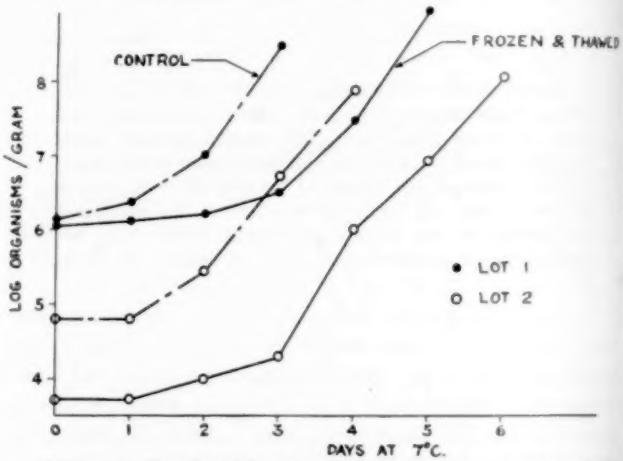

Figure 2. Comparison of growth rates of a psychrophilic bacterium in unfrozen and thawed frozen meat at 44.6° F. (7° C.).

and thawed was compared with growth in unfrozen controls. The frozen portion of each lot was held in the freezer for 2 weeks before thawing. Lot 1 showed very little difference in count before and after freezing but bacterial growth in the unfrozen or control samples began immediately and entered the logarithmic growth phase by the second day. The frozen and thawed tubes of Lot 1, however, had not recovered from the lag phase until after 2 days at 44.6° F. (7° C.), and did not begin exponential growth until after the third day.

Lot 2 showed a marked decrease in count during the 2-week freezing period but is otherwise similar to Lot 1 in the growth patterns at 44.6° F. (7° C.). When generation time for the exponential portions of the four growth curves in Figure 2 was calculated *(6)*, values of 4.81 and 5.89 hours were obtained for the control and

[b] Obtained through the courtesy of Dr. Frank Davis of the Naval Medical Research Institute, Bethesda, Maryland.

frozen and thawed groups, respectively of Lot 1, while 5.99 hours for the control group and 6.84 hours for the frozen and thawed group of Lot 2 were found. It is interesting to note that not only was the lag phase lengthened by freezing and thawing but that the generation time was increased for approximately 1 hour by the freezing procedure. A lot of ground beef inoculated with the psychrophilic test culture showed almost parallel growth curves to the two pork lots and generation times of 4.50 and 5.31 hours, respectively, for control and frozen and thawed groups.

In addition to the results discussed above which show that freezing and thawing tends to lengthen the lag phase and generation time of psychrophilic bacteria, it seemed advisable to investigate the growth, if any, of known mesophilic organisms in thawed frozen meat held in a refrigerator. Figure 3 shows the results ob-

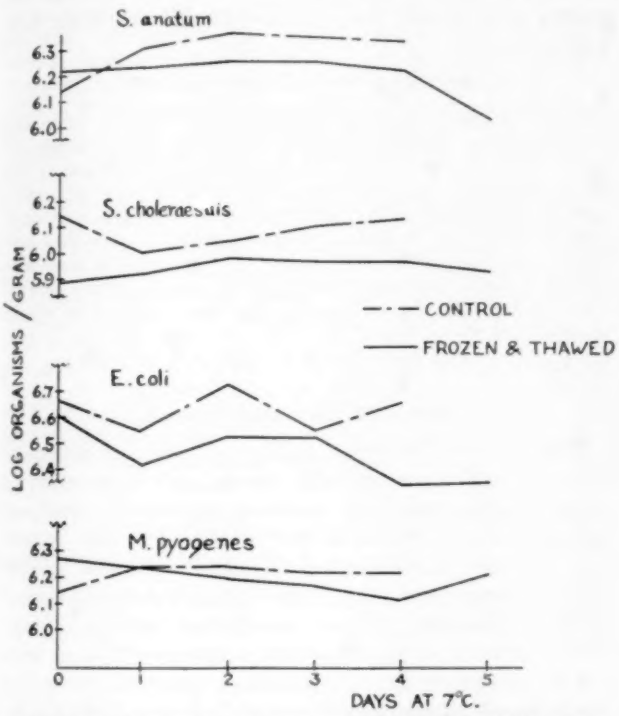

Figure 3. Comparison of counts of known mesophilic organisms in unfrozen and thawed frozen meat at 7° C.

tained with four lots of pork inoculated with *Salmonella anatum, Salmonella choleraesuis, Escherichia coli,* and *Micrococcus pyogenes,* respectively. The broken lines represent the counts obtained from unfrozen samples and the solid lines the counts from samples which had been held in a freezer for 2 weeks prior to thawing and placing in the 44.6° F. (7° C.) refrigerator. Although there is a considerable fluctuation in all the counts there is no clear evidence of growth of any of these organisms in ground meat in the 5 day period. The control lot of *S. anatum* inoculated meat did show a consistent increase in count during the first two days which may have been growth rather than experimental error because this organism has a somewhat lower optimum growth temperature than the others. In all four experiments summarized in Figure 3, the frozen and thawed samples had lower counts than the control lots. In the cases of *S. anatum* and *M. pyogenes,* the slight increase in count immediately after thawing was interpreted as being due to experimental error. The cold stimulation of *E. coli* mentioned above as having been observed by Hartsell *(2)* in eggs was not observed in this experiment. It is to be expected that in experiments such as these four, a rather large experimental error is induced by the fact that the organisms used for inoculation have a higher optimum growth temperature and a different physiology than the usual meat flora. It did not seem unlikely that, at 44.6° F. (7° C.), the normal flora of the meat might even completely obscure the inoculated organisms. However, during the course of the work the plates of each of the four test organisms were observed to differ from each other and before the end of the experiments the plates could be identified by colony appearance so characteristic was the difference. To obtain further evidence that the organisms used for inoculation actually persisted throguhout the experiments slides were made from characteristic colonies on the plates counted and examined microscopically. Comparisons with the original cultures showed them to be morphologically identical in all cases.

SUMMARY AND CONCLUSIONS

1. Psychrophilic organisms inoculated into ground meat while in the logarithmic growth phase showed a prolonged lag phase induced by freezing and thawing the meat.

2. As measured by its generation time, the growth rate of a psychrophilic organism in ground meat that had been frozen and thawed was slower than in unfrozen ground meat.

3. The mesophilic microorganisms, *Salmonella anatum, Salmonella choleraesuis, Escherichia coli,* and *Micrococcus pyogenes* did not grow in either fresh or frozen and thawed ground pork held at 44.6° F. (7° C.) for a period of 5 days.

These results would seem to indicate that there is no scientific basis for the commonly held belief that frozen meat becomes more perishable after thawing than fresh meat. However, the possibility that long periods of freezer storage may give different results from those discussed above should be investigated before drawing final conclusions.

Acknowledgment

The author acknowledges with thanks the valuable assistance of Mrs. Nina B. Hess in conducting the laboratory investigations.

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The Role of Sanitation in Quality Control of Frozen Citrus Concentrates[a]

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The importance of sanitation in the processing of frozen citrus concentrates is stressed. The results of a questionnaire concerning the sanitation practices of juice processing plants in Florida are reported and discussed.

Frozen concentrated juices will have an estimated retail value of $170,000,000 in 1952, making them the largest selling frozen food today. Since at least 91% of all concentrated juices are citrus, their value will reach nearly $155,000,000 *(12)*. To achieve this success in the frozen food field, citrus concentrates have necessarily had consistently high quality and good sanitation practices have definitely played a vital role in assuring that quality.

During the 1945-46 season, when frozen citrus concentrates were first produced commercially, 226,000 gallons were processed while it is estimated that during the present (1951-52) season approximately 42 million gallons will be produced in Florida alone. In addition, California produces between 2 and 4 million gallons of frozen orange concentrate per year as well as a like amount of frozen concentrated lemonade. From its inception, the citrus concentrate industry has laid heavy emphasis on sanitation as an integral part of quality control, primarily because there has been no heat treatment for bacterial control. This tremendous increase in volume has served to point up even more emphatically the necessity for installing the most rigid sanitary practices, including selection of raw materials, careful grading upon their receipt at plants, and the most modern processing methods and equipment. A portion of the industry is now using some form of mild, nonsterilizing heat treatment for partial enzyme inactivation. This has the effect of reducing bacterial hazards slightly but does not decrease the necessity for proper sanitation.

The process for manufacturing citrus concentrates in general use in Florida and California has been described by Burton *(4)* and Beisel *(2)*. It consists primarily of the following steps: a, receiving fruit at the plant; b, grading fruit for soundness and maturity; c, storing each load of fruit in individual bins; d, washing, final grading, and sanitizing fruit as conveyed to process; e, extraction in one of several types of commercial machines; f, finishing juice to remove pulp, seeds, and rag; g, evaporation in single- or multi-effect units at temperatures of 55° to 80° F. (12.8° to 26.7° C.) to remove 75% to 90% of the water; h, mixing the concentrate with fresh juice; i, filling the chilled concentrate into containers; j, quick-freezing the containers and packing into cases; and k, storing at 0° F. (—17.8° C.), or lower, until shipment. All of the above steps are important in the sanitary control of quality, but especially is cleaning of the equipment involved; since concentrate plants operate on a continuous basis during the processing season.

It was believed desirable to determine the problems involved and how the frozen concentrate industry has met the sanitation challenges of production which has increased 177-fold in 7 years. This report presents the results of a survey made in an attempt to obtain that information.

METHOD OF INVESTIGATION

A questionnaire covering in detail 15 broad topics concerning plant sanitation practices was sent to each of the 18 concentrate plants operating in Florida. At the time of preparation of this paper, completed questionnaires were received from 15, representitng about 80% of the industry output. There was no reply from 2 plants and the third company stated that its policy prevented participation in the survey. Questionnaires were also sent to 5 plants in California known to process citrus concentrates, but insufficient information has been compiled to permit summarizing those data at this time. It is expected that a summary of California methods will be made at a later date.

RESULTS AND DISCUSSION

The answers to some sections of the questionnaire were of such a non-uniform nature and represented so many slightly different practices that it was believed impractical to tabulate all the results. For this reason, only selected questions are tabulated with the balance being summarized along with whatever interpretation seemed allowable. Where insufficient information has been collected to permit definite statements as to individual practices in the industry, generalizations are made based on the author's observations.

Plant procedures. The first step in assuring a sanitary product is to make certain that the raw materials are of proper quality. In concentrate processing this requires the rejection of overmature fruit as well as that affected with decay conditions such as "stem end" and "blossom end" rots. Another troublesome condition is alternaria in which the central core of the fruit is decayed without there being definite outward signs of degradation. These decays are all caused by molds but recently bacteria tentatively identified as belonging to the genus *Leuconostoc (13)* were observed in a smear from an outwardly sound Valencia orange found to have alternaria. Organisms of this genus have been isolated from spoiled concentrate *(8, 9 10)* and have been shown to cause spoilage when inoculated into orange juice. Hill and Faville *(11)* have pointed out that oranges may have high bacterial counts without visual evidence of degradation and that acid-resistant bacteria will grow in oranges that were artificially inoculated while still on the tree. These data would indicate that fruit should

[a] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Mich., June 10, 1952.

be given thorough inspection continually, internal as well as external.

As fruit is received, it is graded or sorted in stations where the fruit passes over roller conveyors. It has been found *(13)* that on a grading table 4 feet in width, normal fruit should move no faster than about 30 feet per minute and that at least 2 graders should be located on each side of the table. Under these conditions, fruit should move over a table at a rate no greater than 120 boxes (90 pounds each) per grader per hour for satisfactory grading. If fruit is of less than normal quality it may be necessary to reduce the ratio of fruit to graders by as much as 50%. All unsound or soft fruit is sorted out and routed to the peel bin for processing into cattle feed or return to the supplier. Use of ultra-violet lights, as reported in California, to help in the identification of decay conditions not visible by ordinary light, has not been successful at Minute Maid Corporation, although investigation of this technique is continuing.

Each load of fruit, usually 200-450 boxes, is stored separately in a bin so as to help control blending of fruit to obtain optimum quality. Older bins, of wooden construction, are subject to deterioration and accumulation of mold, requiring frequent cleaning. Especially during warm weather, they are fogged with pyrethrum spray each time they are emptied to keep down the propagation of the indigenous fruit flies, *Drosophila*. Bins made of expanded metal offer greater ventilation and less chance for mold growth. Regardless of construction, they must be cleaned periodically, usually by hand with scrapers and brushes, followed by a highly chlorinated rinse.

Methods of washing fruit vary considerably in the industry but, in general, consist of wetting fruit by low or high pressure sprays and then conveying it over rotating nylon or bristle brushes with application of a detergent. The detergent is washed off and a germicide then applied. Most plants use chlorinated water at concentrations ranging from 12 to 50 p.p.m. usually applying it to the fruit just prior to conveying it to the extractors. Three plants use a quaternary ammonium compound applied as a fine mist, after the brush washers, at about 250 p.p.m. The germicide is allowed to remain on the fruit until just prior to extraction where it is removed by high pressure sprays. Exhaustive tests *(13)* using the method of Wilson *(24)* have failed to show evidence of quaternary compound in orange concentrate. Excellent germicidal results have been obtained but there is still no clear preference between chlorine and the quaternary.

Table 1 shows the results obtained in a test *(13)* in which a hypochlorite solution was applied to one line of fruit feeding the process and a quaternary ammonium compound to another identical parallel line. The chlorine solution was kept at an average concentration of 75 p.p.m. and the quaternary compound, at 250 p.p.m. The disparity of concentrations was due to the desire to evaluate the two materials under certain specific conditions; that is, the quaternary at the regular concentration used for several years and chlorine at the highest concentration reported for that purpose.

TABLE 1

Microbiological load on fruit surfaces (results expressed as numbers of organisms per orange)

Fruit line	Sample	High	Low	Average	Reduction
					%
	Unwashed fruit [b]	13,800,000	240,000	4,650,000	.....
	Series "A" (10 samplings)				
1	Fruit after germicide rinse (250 p.p.m. quaternary)	560,000	32,000	187,250	96.0
2	Fruit after germicide rinse (75 p.p.m. chlorine)	1,180,000	32,000	529,750	88.8
	Series "B" (20 samplings)				
2	Fruit after germicide rinse (250 p.p.m. quaternary)	1,780,000	33,000	611,000	86.7
1	Fruit after germicide rinse (75 p.p.m. chlorine)	1,300,000	26,000	444,500	90.5

[b] Representative samplings made during both Series "A" and Series "B."

Tests were made daily during the experiment using the standard rinse technique common to the industry. While Series A shows a greater reduction of microbiological load with the quaternary, Series B (in which germicide and fruit lines were reversed) shows a slightly greater reduction with chlorine. Further work is planned to evaluate these germicides. These results coincide with the reduction reported by the industry, which ranged from 80%-96%.

Final grading is usually performed just prior to germicide treatment and removes fruit damaged in the bins or during conveying. One plant reported washing fruit before storage in bins, using a germicidal solution of sodium pentachlorphenol and borax. Upon discharge from bins the fruit is exposed to chlorinated water spray but undergoes no further washing. This method is said to reduce decay of fruit in the bins but some believe that storage of wet fruit, even after a germicidal treatment, may actually induce decay.

There are several types of commercial juice extractors all of which are satisfactory from a sanitation standpoint if cleaned properly. While there are many different cleaning schedules during operation, it is believed that a minimum program consists of washing all extractors thoroughly twice each shift, ordinarily every 4 to 6 hours. This usually is done with in-plant chlorinated water ranging from 1.5 to 8 p.p.m. of chlorine, delivered from hoses at normal pressure or from high pressure "guns" operating at 150-400 p.s.i.g. In all, 7 plants reported a program basically like that described above. Also, 6 other plants reported that they rinse extractors every 8 to 12 hours with an alkaline cleaning compound at a concentration ranging from 0.5% to 2.0%. This is followed by steam guns and chlorinated water rinse, the latter as high as 100 p.p.m. chlorine. Of critical importance is the necessity for rinsing juice extraction equipment whenever there is a stoppage in the process of over 15 to 20 minutes, since juice exposed to atmospheric temperatures is subject to microbiological growth, and pulp allowed to dry on equipment is very difficult to remove. Extractors constructed of aluminum must be cleaned only with compounds properly inhibited to reduce corrosion.

Finishing of the juice is generally accomplished with standard screw finishers, though at least 3 plants also use rotating cylindrical screens or reels to strain juice as a first stage of finishing. Schedules for cleaning of this equipment are often dictated by screens clogging and the resulting diminution of juice flow. In general, cleaning will coincide with extractor cleaning and is ordinarily of the same order. At least one plant does not remove screens except from the finisher supplying pulpy cutback juice, high pressure guns being relied on

for cleaning the finishers supplying evaporator feed juice. Under certain fruit conditions, which are not yet clearly understood, there is heavy accumulation of a grayish-white crystalline deposit on juicing equipment, generally believed to be composed partially of hesperidin, the naturally occurring glucoside of oranges. Tangerines seem to cause these deposits even more than oranges. This deposit is difficult to remove but is usually susceptible to removal by alkaline cleaners.

Generally, juice troughs, lines, and tanks are also cleaned on a schedule similar to extractors. Two plants clean juice tanks once each 24 hours while another cleans them every 2 hours. Seven plants clean all lines and tanks twice each shift of 8 or 12 hours and 3 reported cleaning them once each shift.

Evaporators, while no more important in the sanitation program than juice extractors, warrant the major consideration in a cleanup program since they a, are the largest piece of equipment in contact with juice; b, are part of a completely closed system; c, operate without cleaning for the longest period of time; d, are more difficult to design for good flow characteristics; e, are fairly inaccessible; and f, operate under temperature and pressure conditions which are favorable to growth of certain organisms known to cause spoilage in orange juice. The latter point is especially important since there has been reported *(9, 16, 20)* identification of strains of microorganisms isolated from spoiled orange concentrate which grow well at soluble solids concentrations up to 35° Brix and at temperatures as low as 55° F. (12.8° C.) and prefer low oxygen tension.

The usual procedure for evaporator cleanup consists of circulating water immediately after pumping out the concentrate and following the water with a caustic solution. Plant practices vary in regards cleaning solutions since there were reported cleaning material concentrations ranging from 0.5% to 10.0% and solution temperatures from 72° to 200° F. (22.2° to 93.3° C.). The average solution would appear to have a temperature of 140° F. (60.0° C.) and a cleaning compound concentration of about 3.0%. Compounds containing sequestering and wetting agents are used at concentrations of about 0.5%. Water hardness, reported as ranging from 7.3 to 19 grains per gallon, is usually reduced to 5 to 10 grains per gallon by means of water conditioners.

After circulating the caustic solutions for approximately 30 minutes, evaporators are often opened in order that personnel may use hoses to wash spots not completely cleaned. This is followed by chlorinated water having a concentration ranging from 1.5 to 400 p.p.m. which is circulated for about 15 minutes to sanitize all surfaces.

Timing of evaporator cleanup varies with such factors as bacterial counts, hesperidin buildup, and loss of evaporation efficiency, but schedules have been reported such as cleaning every 66, 72 or 100 hours, fourth day and sixth day. The mode is 72 hours, which cycle is recommended by many as being the safest from the standpoint of preventing bacterial spoilage. At least 2 plants stagger cleanups of evaporators so as to have no break in product output. In those plants general cleanups occur less frequently and the various units of operation are cleaned separately. When all evaporators are cleaned simultaneously it is necessary to have more frequent general cleanups.

Where a batch process is used, mixing of the concentrate and fresh juice is almost universally accomplished in cold wall tanks, either direct expansion or brine cooled. Three plants who use continuous blending have small unrefrigerated blending tanks and maintain very small volumes of blended product in process. Mixing tanks are washed on schedules such as "after every batch," every 4 to 6 hours, each shift and once every 24 hours, with the average appearing to be once per shift. Four plants reported using caustic solutions for washing periodically, such as every 12 hours, with in-plant chlorinated water rinses between caustic washes. One plant reported that since tanks are refrigerated, interiors of tanks are not washed intermittently, only outside surfaces. Can closing and filling machines are also generally cleaned once each shift. Containers for product are subjected to a jet of steam just prior to filling the container with product in order to remove foreign matter and partially to sterilize the interior of the container.

Almost all plants in Florida have quick-freezing facilities, either air blast or brine immersion types. The freezing and storage of the product are important since DuBois and Kew *(5)* have shown that the product must be maintained at temperatures of 0° F. (—17.8° C.), or lower, for satisfactory preservation of quality. Since Faville and Hill *(6)* have shown that microorganism counts may be decreased by 80%-90% during storage at 0° F. (—17.8° C.) or —10° F. (—23.3° C.), it is apparent that there will be a decrease in counts while the product is held at proper temperatures, either at the plant or in the field.

Only two companies have facilities for testing and evaluating insecticides, but 4 others have conducted tests in cooperation with insecticide manufacturers. In general, pyrethrum sprays are utilized inside the buildings where fruit or juice is processed and in bins where fruit is stored. Various other materials are used in outside areas including Thanite, chlordane, lindane, and cube extractives. No data are available as to usefulness of specific outside sprays but it is generally recognized that houseflies found in Florida are particularly resistant to chlorinated hydrocarbons *(7)*. Minute Maid Corporation has undertaken a cooperative experiment with the manufacturer[e] of a new unnamed synergist used in conjunction with lindane for outside space-type spraying. This material is used in a water emulsion spray having a final synergist concentration of 0.5% and a lindane concentration of 0.5%. It has not been completely evaluated yet but preliminary reports are promising. While the point was not covered by the questionnaire, it has been observed that, in general, all concentrate plants make a strong effort to keep juice preparation areas screened for insect protection. The only other area where the product is exposed is the packaging room which is usually totally enclosed in some manner.

Control of rodents is not usually considered a serious problem in concentrate plants because there is little storage of materials which attract rodents and little possibility for contamination of products; nevertheless, rodents will occasionally eat citrus fruit and dried citrus pulp used as cattle feed. In addition, containers for

[e] California Spray-Chemicals Co., Orlando, Florida.

products must be protected, making necessary some definite precautions. While not revealed by the questionnaire, a later check revealed that all plants have a control program but are split fairly evenly between outside commercial control and their own plant agencies. Warfarin is almost universally used in these programs, whether company organized or commercial.

Laboratory control methods. Several techniques are employed in determining just when evaporator cleanup should occur, including examination of product for excessive hesperidin and estimation of loss in evaporation rate. Probably the most widely used, however, is the estimation of microbiological population by means of the total plate count. All plants but one indicated use of "orange serum agar" *(10, 15)* at a pH ranging from 5.4 to 6.0 as a medium for total counts. One plant makes no plate counts, relying entirely on other methods. The final product sampling schedule depends on method of operation, whether by batch or by continuous blending. All plants but 3 operate by a batch blending method and 7 sample at least one can from each batch, while 2 plants reported sampling only odd-numbered batches (Table 2). The 3 plants reporting a continuous blending of concentrate and cutback juice, sample product each hour for total bacterial count. No information was obtained as to interpretation of counts in regard to quality of product or use as an index of plant sanitation but data have been presented by Patrick *(17)* to show that bacterial counts at time of production may range normally from 3,300 to 400,000 per ml. of reconstituted juice. So far as is known, there is no direct correlation of total counts with flavor *(17)* but a steady rise or sudden increase in counts is generally taken to indicate an unsatisfactory condition in the process.

TABLE 2

Questions and answers in sanitation questionnaire for laboratory control procedures. (Numerals indicate number of plants.)

Question No.	Questions and answers	
1	What is your frequency of sampling canned products for bacterial plate count (in terms of hours or batches, whichever applicable)?	
	Each batch	7
	Every other batch	2
	Hourly	3
	Every 3 hours	1
	None	1
2	Do you regularly make differential counts on canned product, or do you rely on total counts with one medium?	
	Total counts	5
	Differential counts	9
3	Do you test for presence of coliform bacteria?	
	Yes	9
	No	5
4	Do you feel that line checks are a definite help in maintaining desired sanitation standards?	
	Yes	14
	No	0
5	Do you use microscopic examination of juice as a control procedure?	
	Yes	6
	No	3
	Not regularly	1
6	Have you been able to correlate microscopic counts with (a) plate counts?	
	Yes	0
	No	7
	(b) spoilage conditions?	
	Yes	5
	No	1
	Occasionally	1
7	Are bacterial counts and other pertinent data transmitted to top production personnel on regular basis?	
	Yes	11
	No	3

Location of sampling points for bacterial counts on juice in process (commonly called "line checks") vary considerably but usually include juice from the extractors, juice troughs or lines from extractors; juice after finishers, from tanks and various stages in the evaporators; concentrate from blending tanks; cutback juice; and final canned product. A few plants include estimation of bacterial population on fruit surfaces before washing, after washing, after sanitizing, and before extraction. These line check samples are taken on schedules such as once every 2, 8, 12, or 24 hours. All plants utilize some sort of line check procedure and the usual frequency is once per shift. All plants stated that they believe this information valuable as part of a sanitation program.

The 10 plants making plate counts use at least one other medium to help differentiate known spoilage type bacteria from total microbiological population. While there have been no exact data published to show that media now used are completely reliable for growth of specific bacteria causing spoilage, there has been evidence that counts on sorbic acid-orange serum agar *(9, 20)* do increase regularly with increase in population of *Leuconostoc* and *Lactobacilli*. This medium has been adapted from one suggested by Vaughn *(21)* for detection of catalase-negative organisms and its use was reported by 5 plants. McCleskey's sucrose agar *(1, 14)* is used by at least 5 plants for indication of numbers of gum-forming organisms, particularly of the genus *Leuconostoc*. Differential media are used regularly for samples from specific locations in the process, particularly early stages of concentration since both genera mentioned above grow best at soluble solids concentration below 35° Brix.

Another technique which has found extensive use during the 1951-52 season is the estimation of bacterial population by means of microscopic examination of stained smears of concentrate or juice. Several methods of staining have been suggested but the one reported by most plants was adapted from that of North *(18)*. Twelve plants stated that a microscopic examination was used as part of their control programs but none could find a definite correlation with plate counts. Six reported some correlation with "spoilage conditions" though the questionnaire did not ask for more details. It has been found *(13)* that microscopic examination of concentrate from intermediate stages of evaporators made every 4 or 8 hours can definitely be interpreted, on the basis of experience in a particular plant, to determine when evaporator cleanups should occur so as to prevent excessive bacterial buildup. One plant relies completely on microscopic examination and makes no plate counts. The microscopic method as generally practiced is not believed completely reliable and must be judged in relation to other factors; nevertheless, it has the most attractive possibilities as a rapid control procedure of any presently available. A chemical method *(23)* for

detection of acetyl-methyl-carbinol or diacetyl, metabolic products of certain acid-resistant bacteria causing spoilage, shows promise but has not yet been made sensitive enough to be relied upon. The phase contrast microscope may be adaptable for rapid estimation of bacteria in orange concentrate and this instrument is being studied extensively by several firms in Florida.

Nine plants reported that regular tests are made for coliform organisms, using the Vaughn-Levine boric acid broth *(3, 22)* as a presumptive medium. Three plants make the test hourly while the others reported sampling each batch. The latter group usually composites these batch samples during a shift and then takes a sample from the composite for the actual presumptive test. Most concentrate plants utilize eosine methylene blue agar in confirmatory tests as well as portions of the IMViC pattern while 3 plants use the Eijkman lactose medium *(19)* as well. Three plants reported that product showing positive confirmatory tests is held and retested at monthly intervals until negative. Another organization stated that special attention was accorded such product but did not explain further.

Regular examinations of product for mold and insect contamination are made by inspectors of the U.S.D.A. Production and Marketing Administration who inspect all processed citrus product in Florida. These tests are particularly helpful in determining condition of fruit and, of course, are a standard index of sanitation in the eyes of the Food and Drug Administration.

Organization and administration of the sanitation program. A few of the questions and answers dealing with this subject are listed in Table 3.

TABLE 3

Questions and answers in sanitation questionnaire for the organization and administration of sanitation program. (Numerals indicate number of plants.)

Question No.	Questions and answers	
1	Who has the responsibility for formulating sanitary standards?	
	Quality Control	10
	Quality Control and Production	3
	Production	1
2a	Are these standards issued by the above person?	
	Yes	11
	No	0
2b	Do they then have official standing as company policy?	
	Yes	10
	No	0
3	Does the person responsible for issuing standards also have the responsibility for outlining methods to accomplish the standards?	
	Yes	11
	No	0
4	Who has the direct responsibility for maintenance of sanitation at the level prescribed by company policy?	
	Quality Control	8
	Quality Control and Production	4
	Production and Maintenance	2
	Each Department	1
5	Do you have a separate Sanitation Department?	
	Yes	2
	No	13
6a	If there is no separate Sanitation Department, are the responsibilities and duties of sanitation supervision and instruction incorporated in your Quality Control Department?	
	Yes	10
	No	1
	Partially	3
6b	How is the responsibility delegated?	
	Quality Control	7
	Quality Control and Production	2
	No Definite Pattern	1
7	Do you have a formalized sanitation program?	
	Yes	9
	No	4
8a	Does this program include instruction of supervisors and employees?	
	Yes	12
	No	2
8b	Is this instruction carried out on company time?	
	Yes	11
	No	0
9a	Does this sanitation program include an outline of specific plant housekeeping?	
	Yes	12
	No	1
9b	Does this program include ground care and maintenance?	
	Yes	11
	No	1
	Insofar as Pest Control	2
9c	Does this program include an outlined insect control procedure?	
	Yes	13
	No	1
9d	Do you have facilities for and carry out insecticide evaluation?	
	Yes	2
	No	8
	Limited	4
10a	Does this sanitation program include an outlined and definite procedure for cleaning during operation?	
	Yes	14
	No	0
10b	Does this sanitation program include an outlined and definite procedure for general clean-up?	
	Yes	14
	No	0
11a	Do you have a sanitation rating system for operation and clean-up?	
	Yes	6
	No	6
11b	Is there an incentive factor attached to sanitation ratings?	
	Yes	5
	No	5
11c	Who inspects and rates sanitation during operation and clean-up?	
	Quality Control	8
	Quality Control and Production	2
	Production	1
12	Is there a schedule for formal inspection during operations?	
	Yes	8
	No	5
13	After general clean-up, do you require inspection of equipment by Sanitation representative prior to resuming operations?	
	Yes	13
	No	0
14a	Is the actual clean-up handled by Production personnel?	
	Yes	12
	No	2
	(1 "Yes"—Except Juice Room and Evaporators)	
14b	If answer to 14a is "no," do you have special clean-up crews?	
	Yes	2
	No	0
14c	If so, just how is special crew set up and scheduled?	
	4-man volunteer crew in juice room; 2-man volunteer crew on evaporators; on-time bonus plan	1
	Under direction of leader man, who is responsible to Production	2

In general, setting of standards and general supervision of sanitation are delegated to Quality Control personnel. Most plants have some definite program to

implement the sanitation standards and give instruction to employees on company time. Only 6 have a system for rating the plant in its sanitation efforts but 5 of those attach a wage incentive factor to the ratings.

CONCLUSIONS

It was concluded from the survey that sanitation is playing a vital role in the control of quality of citrus concentrates. In the manufacture of concentrates each step, from harvesting of fruit to storage of finished product, is important and all plants seem to have a fairly adequate program for controlling sanitation at those points. The experience of the industry is that high quality products are consistently produced and this fact would tend to confirm the adequacy of sanitation practices. The uniformity of approach to equipment cleaning problems among the various plants is partially explained by the striking similarity of process and equipment used by all plants. The organization and administration of the sanitation program seems to follow a pattern involving delegation of policy making and supervision to quality control personnel but in many cases production personnel have the responsibility for maintenance of the standards. The principal sanitation problem facing concentrate manufacturers appears to be microbiological control. At present that problem is being met by continuous testing of juice and product by one or more methods to detect conditions which might result in poor quality of the concentrate. This routine inspection is valuable as a control measure but does not get at the sources of the problem, which are the normal bacterial contamination of commercially extracted juice and the favorable growth conditions found in most evaporators. Possibly there is no method short of pasteurization which will allow establishment of positive control over the process and that would be undesirable from a flavor consideration; however, the mild heating now practiced by some manufacturers will probably give a greater margin of safety than existed previously. It is suggested that additional research is needed regarding factors influencing internal quality of fruit, as well as to improve processing methods and test procedures since continued growth of the industry will require even better sanitary controls.

SUMMARY

The frozen concentrated citrus juice industry has increased 177-fold in 7 years to an estimated dollar sales volume in 1952 of $155,000,000. This increase in production of a product normally not heat-processed for control of bacterial population has required establishment of rigid sanitation standards to maintain quality. A questionnaire answered by 15 concentrate plants in Florida has revealed that sanitation practices are at a high level and that management, in general, is very conscientious in maintaining these standards. It is believed that all plants stress proper harvesting procedures and control of quality of fruit utilized. Fruit surfaces are sanitized to reduce the bacterial load by 80% to 95% and all juice preparation equipment is cleaned frequently during operation. Evaporators are cleaned with caustic materials and germicides at intervals ranging from every 66 hours to every sixth day. Plate counts and microscopic examination of juice are used to indicate bacterial levels and proper timing of cleanups. Insect and rodent control practices appear to be adequate. Most plants have sanitation supervision responsibilities delegated to the Quality Control Department. Production personnel are ordinarily charged with maintenance of sanitation standards and plant cleaning.

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Examination of Citrus Juices Processed from Parathion-Sprayed Fruit[a,b]

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Citrus juices from fruit sprayed with parathion for the control of citrus insects were canned and concentrated. Analyses of samples of these processed products showed that most of the packs contained no parathion. Minute quantities were detected in a very small percentage of the total packs processed. No off-flavors which could be attributed to parathion were detectable.

Since parathion is being used widely for the control of citrus scale insects *(4)*, an investigation was made to determine whether any parathion would be introduced into and remain in citrus juices after extraction and processing into canned products and frozen concentrates. Prior work *(7)* had shown that parathion can penetrate into the peel of the fruit, but is not found in the juice sacs. It was further established that parathion is concentrated in the peel oil *(5)*. However, since the amount of peel oil found in processed citrus juices is extremely small, there is little likelihood of these processed citrus products presenting a health hazard. Nevertheless, since over 55% of the citrus fruit produced in Florida eventually is sold as a processed product, it was deemed advisable to establish definitely whether processed products made from parathion-sprayed fruit would contain parathion and whether off-flavors would occur in these products either initially or during storage. Griffiths, Reitz, and Olsen *(3)* reported that no off-flavors developed in single-strength canned orange juice made from fruit sprayed with parathion. During this same investigation juice from fruit sprayed with benzene hexachloride, chlordane, or toxaphene was canned also, and off-flavors were found only where benzene hexachloride had been used.

Citrus production and processing facilities at the Florida Citrus Experiment Station made possible the investigation described in this article. This work illustrates what can be done to obtain the much-needed factual information regarding the presence or absence of harmful residues when toxic insecticides are used on certain food products.

EXPERIMENTAL PROCEDURE

All of the oranges and grapefruit used were obtained from Station groves and the amount of parathion used in sprays was carefully controlled. In most cases an exceedingly heavy application of parathion consisting of 0.3 pound per 100 gal. was used in each of three applications. This amount is much larger than that recommended and also beyond a practical commercial range. However, it was thought that it was much better for the purpose of this work to exceed the limits of commercial practices.

After the fruit was thoroughly washed in accordance with standard practice the juice was extracted, using four different commercial extractors commonly employed in the citrus industry. Use was made of the extraction facilities of two neighboring canning plants where Brown extractors and Food Machinery Rotary Juice extractors were available. A Bireley Citro-Mat and a Clinton Foods Rotary Juice Press installed at the Station were used also. All of the extracted juices were canned or concentrated, using processing equipment available in the pilot plant at the Station. A short-time, high-temperature Mojonnier pasteurizer and a low-temperature, high-vacuum evaporator, previously described *(1)*, were used.

The parathion content of the freshly extracted citrus juices was obtained by taking samples from 60 gal. stainless steel tanks after thorough mechanical mixing had taken place. Then 200 g. of the juice sample was put into a 1-liter cylindrical separatory funnel and 1 to 2 g. of benzene per g. of juice was added. The funnel was securely stoppered and placed in a horizontal shaker for 1½ hours. The extraction should be started and completed as soon as possible in order to avoid excessive emulsification that apparently is caused by low-methoxyl pectin developed by enzyme activity in the juice. After the extraction period, time was allowed for separation of the two liquids. The juice or bottom layer was drawn off and the benzene extract stored in a well-stoppered flask. If a tight emulsion formed it was broken by use of centrifugal force.

Then 100 g. of the benzene extract was weighed into a 300-ml. tall-form beaker and decolorized according to the Averill-Norris *(2)* procedure, using 11 g. of clay-supercel mixture. The clay was washed with two 25-ml. portions of benzene. The total sample was transferred back to the beaker and the filter flask was washed with 25 ml. of benzene which was added to the sample. From this point the Averill-Norris procedure was followed. This method gave about 80% parathion recovery. With processed single-strength juice a composite was made from five 9½-ounce cans and a 200 g. sample was taken. Three 6-ounce cans of 42° Brix concentrate were reconstituted and well mixed before withdrawing a 200 g. sample for analysis. The analytical procedures for the single-strength canned juice and the frozen concentrated juice were identical to that used for fresh juice.

RESULTS AND DISCUSSION

Orange juice extracted from the same lot of fruit by each of the four commercial extractors showed no significant difference in parathion content. The range in parathion content of 23 batches of freshly extracted juice was between 0 and 0.20 p.p.m., with only four batches showing the presence of parathion. Since the parathion content of these juices was so low (if any), it was in most cases unnecessary to process the juices to determine final parathion content in the finished products. Nevertheless, they were canned and concentrated and when these processed products were analyzed the parathion content was between 0 and 0.15 p.p.m. None of these finished products initially and after storage for 1 year and none of the raw juices had any off-flavor due to parathion.

To determine what loss of parathion may occur during processing an oil emulsion of parathion was intentionally added to orange juice, which was then processed into single-strength canned juice and frozen concentrate. Analyses of these processed products dis-

[a] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Michigan, June 9, 1952.

[b] Cooperative research project of the Florida Citrus Experiment Station and the Florida Citrus Commission, Lake Alfred, Florida. Florida Agricultural Experiment Station Journal Series, No. 77.

closed wide variations in the amounts of parathion lost. During the processing of single-strength juice 24 to 48% was lost and in concentrating the loss of parathion ranged from 6 to 25%.

From results obtained it is quite evident that the use on citrus fruit of no more than three sprays of 0.3 pound per 100 gal. of parathion will neither lower the quality nor result in harmful concentrations of parathion in either single-strength canned products or frozen concentrated citrus juices. It is obvious from the data presented that the minute quantities of parathion found in only a small percentage of all of the citrus products processed during this investigation are considerably less than the suggested tolerance in foods of 2.0 p.p.m. of parathion *(6)*.

SUMMARY

Processed citrus juices and frozen concentrates produced from parathion-sprayed fruit were found in most cases to contain no parathion. In the few instances where parathion was detected in such products the minute quantities present caused no off-flavor and could not be considered harmful to consumers.

Acknowledgment

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Toxicity Testing of Chemical Additives[a]

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(Manuscript received June 20, 1952)

The steps involved in testing the toxicity of chemical additives are described and illustrated. The need for more rapid testing methods is reported and a possible solution for some cases is described.

The tools of the toxicologist are adequate, if properly utilized, to protect the public health against undue hazard from chemical additives. By determining the physiological response to a given chemical in several species of laboratory animals the toxicity of the substance is determined. The toxicologist thus carries out biological studies to determine the capacity of the test substance to produce injury.

Such toxicological data can then be evaluated in the light of human exposure. (Exposure is determined by analysis of foods for pesticide residues and by a study of use levels of intentional additives to determine the hazard involved in the proposed use of the chemical.) In other words it is necessary to have data both on human exposure and toxicity before one can decide what the probability is that a given chemical will cause injury when used in the quantity and in the manner proposed.

[a] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Mich., June 9, 1952.

The Food Protection Committee of the National Research Council has issued a statement entitled: "Basic Principles Involved in the Use of Chemical Additives in Foods." These guiding principles at the moment seem adequate to evaluate the toxicity data that may be obtained on any proposed chemical additive.

The tools the toxicologist can employ to obtain the data necessary for arriving at a judgment of the hazard involved in the use of chemical additives are discussed below. The amount of data required for a new additive may well be greater than for a new drug. Two major types of hazards exist: 1, hazards involved in the handling of chemicals in the manufacturing plant and later in their application; and 2, hazards of chemical additives in foods, whether intentional such as coloring or flavoring agents, or incidental such as pesticide residues. It is with the second category that we are concerned and we must keep clearly in mind the distinction between the two.

The first step in procuring toxicological data on a new chemical involves the search for any acute effects that may result from the administration of the test substance. "Acute tests" are "quick tests" wherein a measured quantity of the chemical is injected directly into the body of the test animals (rat, mouse, rabbit, etc.) by one of several routes (intravenous, oral, intraperitoneal, etc.). Salient features are: a, a quick

answer from single or multiple dose; b, use of relatively small numbers of several animal species in screening tests; and c, determination of firm LD_{50} value. LD_{50} may be defined as the dose to kill half the animals in a given time period; or the dose that will kill the average animal. The LD_{50} is derived from the dosage-mortality curve, which is a measure of variability in response. A flat curve indicates occasional instances of extreme sensitivity; a steep curve, little variation. In Figure 1, percent

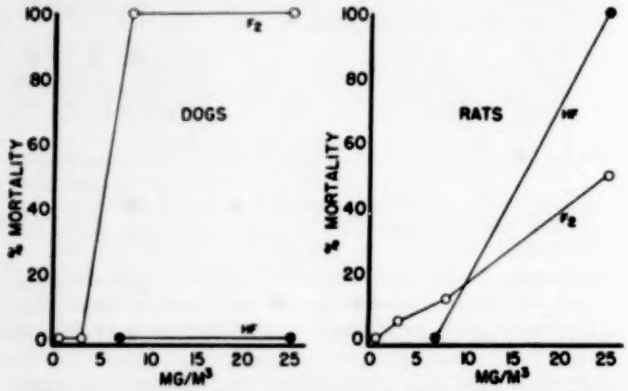

Figure 1. Dosage-mortality curves for fluorine and hydrogen fluoride in dogs and rats.

mortality is plotted against dosage by inhalation of two test materials, fluorine (F_2) and hydrogen fluoride (HF). With F_2 the dosage-mortality curve is steep in dogs but rather flat in rats; with HF the curve in dogs is absolutely flat but in rats the curve is very steep. Such variation in response between species should be viewed with concern for the type of response one might expect in man. The LD_{50} value may then be compared with LD_{50} values of chemicals of known toxicity, to place the test substance in its proper toxicity class. Hodge and Sterner *(1)* have published a tabulation of toxicity classes shown in Table 1.

TABLE 1

Toxicity classes with probable lethal doses (1)

Toxicity rating	Category	Single oral dose	Probable lethal dose for man
1	Extremely toxic	1 mg. or less per kg.	A taste 1 grain
2	Highly toxic	1—50 mg.	1 tsp. 4 cc.
3	Moderately toxic	50—500 mg.	1 oz. 30 g.
4	Slightly toxic	0.5—5 g.	1 pint 250 g.
5	Practically non-toxic	5—15 g.	1 quart
6	Relatively harmless	15 g. and more	>1 quart

Frequently chemicals are eliminated from further consideration for a particular use if they prove to be highly toxic or if the test animals show widely variable responses. (The toxicity per se should not alone rule out the possible use of a chemical. Furthermore we should eliminate the term poison per se from our thinking and from regulatory measures wherever it may now exist. To the toxicologist there is no such thing as poison per se.)

The second stage of a toxicological testing program involves pilot feeding studies. These usually are of 2 to 4 weeks duration, using small groups of rats (5 males per group) with controls and 3 test levels mixed in the diet. Criteria used are growth and mortality. One thus determines the lethal or the toxic range, if any, and acceptability of the material in the diet. One or two dogs are frequently used also.

Then follow short-term studies for 30-90 days using groups of 10 male and 10 female rats, at 3 or more dietary levels. In addition 2 or 3 dogs are ordinarily studied. Criteria studied include growth, mortality, terminal hematology, organ weights, and histopathology. From such studies one establishes the toxic level and the nature of toxic responses and the tissues effected. These data also permit selection of suitable dosage levels for long-term studies. Figure 2 shows typical average growth curves for a short-term experiment in which groups of male rats (10 per group) were fed a test substance at various dietary levels for 7 weeks.

At this point in the testing program a decision is usually reached on the question: "Is the product a good bet?" An overall evaluation is made of such items as: 1, economy of production; 2, efficiency of the product for the proposed use; 3, safety of handling; and 4, the probable margin of safety. If the go-ahead signal is given, long-term feeding studies may then be carried out.

Chronic feeding studies usually involve life-time experiments (2 years) with large groups of rats. Dogs (several per group) are usually fed the test substance for 1 year or more. In both

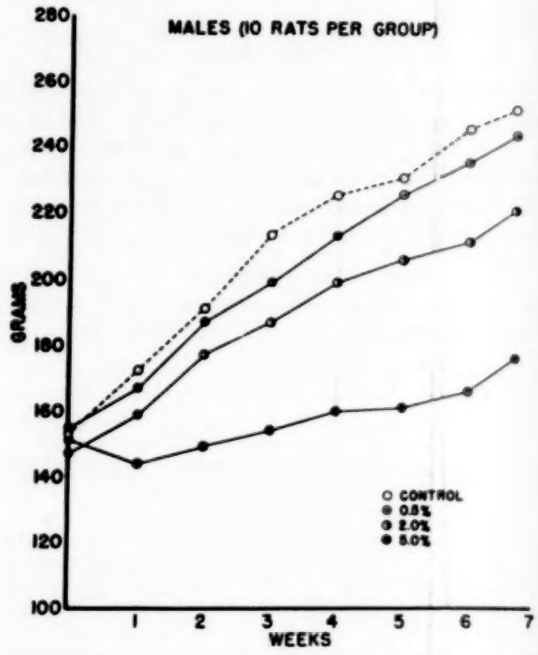

Figure 2. Typical average growth curves for a short-term experiment. Dietary levels of test substance are 0.5, 2.0, and 5.0%.

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species the additive is fed at 3 dietary levels. Criteria of toxicity may include growth, mortality, food consumption, paired feeding, biochemical studies of blood and urine, reproduction (generation) studies, hematology, organ weights, histopathology serially or terminally only, and tissue analyses. (One of industry's most important considerations should be the development of an analytical method for quantitative determination of each new material.) Life-time experiments provide time for development of delayed effects, such as cancer.

The interpretation of the animal data obtained in such a testing program in terms of hazard to man poses a serious problem. In this instance, experience is very important in rendering judgments and perhaps we are now reaching the point in the development of toxicology where we have sufficient experience in the form of accumulated data to permit reasonably sound judgments for the first time.

A program of toxicity testing such as I have so briefly outlined has been subject to much criticism because it is time consuming and costly. As yet, to my knowledge, no suitable substitute has been proposed. I have long been firmly convinced that such a complete program of testing is not always necessary to obtain sufficient data to judge the probable hazard to man. Certainly all of us in the field of toxicology are gravely concerned (or

should be) over the high cost of conducting toxicity experiments, and many of us are searching for less expensive ways of getting the answers. (In all of his endeavors in this direction, however, the toxicologist must remember he has a moral obligation to protect the public health at any cost.)

Toxicology is relatively a new science and it was inevitable, it seems to me, that many inadequately tested chemicals were added to foods in the past. A good many of these additives are still in use and perhaps we shall not really know about the hazards involved until many more years have passed. If the entire list of such substances were scrutinized it is probable that only a few would be suspected of being hazardous as used. These few should be carefully investigated. I doubt that facilities could be provided in any reasonable time (except at tremendous expense) for testing all additives now in use. Moreover I do not suggest that this is necessary. Nor am I alarmed over the many new additives being proposed for I feel that adequate precautions are being taken to insure safety within reason.

Much of the "champing at the bit" on the part of industry today is because of the long lag between the first stages of the development of a new chemical and the ultimate determination of its use hazard. To me there appear to be two alternative solutions to this problem and we must squarely face the issue. These two alternatives are: 1, slow down the rate of application of new additives in foods; or 2, speed up the toxicity testing program. It is obvious that either alternative will be costly; and how to protect the interests of the "little fellow," I don't know. (Some one must find this answer in order to perpetuate individual enterprise.) What industry must do, it seems to me, is face the issue squarely and decide which of the two courses will impede progress the least. Costs must be balanced against values here also.

All industries involved in this problem should join forces with those toxicologists already at work (both within and outside industry) in the development of quicker and less costly methods.

Can substitutes be developed for the long-term feeding studies? In some instances the answer definitely is yes; in many cases probably no.

What kinds of short-cuts can be taken? For example let us say that long-term studies have demonstrated a low order of toxicity for a group of closely related chemical compounds, and a new compound in the same series is ready for testing. If acute and short-term studies place this substance in the same toxicity class with the others and in addition it can be shown experimentally that this material is not absorbed from the gastrointestinal tract, it would certainly seem unnecessary to conduct the long-term experiments.

At Rochester an experiment *(2)* was recently carried out by the expedient of labeling with radioactive sulphur[35] the sulfate groups in a cellulose derivative, sodium cellulose sulfate (SCS). A study of the absorption and excretion demonstrated that insignificant quantities of sodium cellulose sulfate were absorbed from the gastrointestinal tract of cats. Cats were used because other workers had previously demonstrated a similarity between man and the cat in their inability to absorb cellulose.

In Figure 3 the percent of dose excreted in urine is plotted against time in hours. The upper (control) curve shows that

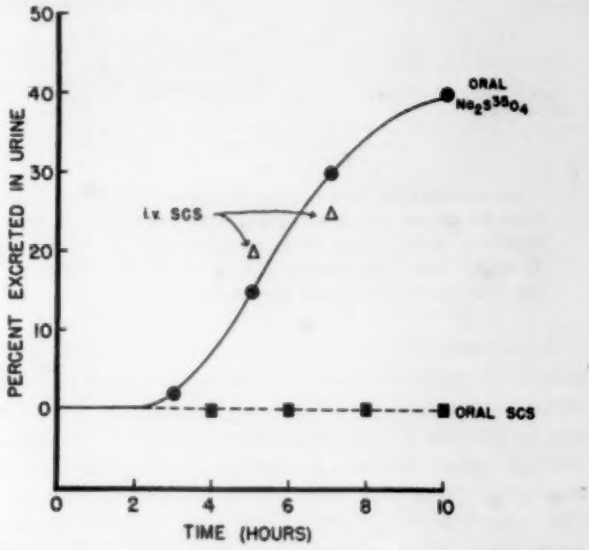

Figure 3. Percent of test dose excreted in urine when administered in different forms.

when inorganic sodium sulfate labeled with S^{35} was given orally, up to 40% of the activity was excreted in a few hours. The points indicated by the arrows show that after intravenous administration of S^{35} labeled sodium cellulose sulfate (SCS) considerable activity (up to 25%) was recovered from the urine within 7 hours, demonstrating that activity of labeled sodium cellulose sulfate would be found if sodium cellulose sulfate got into the blood. The lower curve shows that following oral administration of S^{35} labeled sodium cellulose sulfate there was only insignificant urinary excretion demonstrating insignificant absorption from the gastrointestinal tract. Had there been splitting of the sulfate one would have expected the curve of excretion to be approximately the same as for the inorganic sodium sulfate. Had sodium cellulose sulfate been adsorbed intact, some sodium cellulose sulfate would have been excreted. Counts on tissues from the cats confirmed the lack of absorption.

The relative cost of this experiment was about 1/10 to 1/5 that for the chronic feeding studies. Time saved was about 2 years.

I have faith that this type of study as well as carefully conducted metabolic studies of various types, when coupled with short-term studies to demonstrate lack of irritation or of acute toxicity, may frequently be expected to give quicker and less costly answers. We all would do well to bend every possible effort toward the development of such methods.

In summary, I believe that the toxicologist has the tools to protect the public health against undue hazard in the use of chemical additives. Although the monetary cost is high, we must never lose sight of our moral obligations. We should all strive to develop more efficient toxicological methods as an answer to the too-often repeated charge, "impeding progress of science." Toxicological testing is a mighty important part of that very progress of science and it behooves all of us to assign to it its proper value.

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An Accelerated Cure for Bacon[a]

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(Manuscript received February 6, 1952)

An accelerated cure (18 to 30 hours) for bacon has been developed which compares very favorably in palatability with 21-day, dry, box-cured bacon. The effects of time, temperature, and degree of pickle on the quality of the bacon are reported.

Top-grade bacon produced by the meat packer usually requires a 21-to-28-day cure. Such a lengthy process requires the packer to keep large inventories and to provide a large amount of expensive refrigerated storage space. Packers in their research programs have attempted numerous ways of expediting bacon curing. The experiment reported herein was conducted to develop an accelerated cure for bacon (18 to 30 hours), to compare this bacon with that produced by the nonpressed box-cure bacon which requires at least 21 days in cure, and to provide more information as to the effect of heat upon the length of curing time.

An extensive study *(3)* to determine the need for bacteria and nitrate in the bacon curing process resulted in the following conclusions: 1, bacon with good color and flavor could be obtained from the use of nitrite in a magnitude as low as 10 p.p.m. of lean; 2, the cured flavor of bacon was due primarily to the action of nitrite on the flesh and a satisfactory bacon can be made by using only sodium chloride and sodium nitrite; and 3, the presence of microbial action was not essential for the development of bacon flavor.

Hence, it appeared that time was not necessary for the development of flavor or color if the desired chemical reactions were completed. If bacon is cured at temperatures low enough to inhibit bacterial growth, the speed of chemical reactions, diffusion, osmosis, and capillary movement also is retarded. It seems possible to accelerate considerably the cure of bacon by raising the temperature to approximately 120° F. (48.9° C.), which is low enough to prevent excess melting of fat, yet high enough to inhibit bacterial growth.

Although meat packers have referred to the many attempts to hasten the curing of bacon, very few of these have been published or registered as patents that later were successful processes. Coffman *(4)* introduced a multiple needle injection apparatus to be used in curing bacon. This apparatus had approximately 100 fine, hollow needles and injected small, but equal amounts of cure solution from each needle into the green belly. The belly was stored for 24 to 48 hours and smoked as in other conventional methods. Komarik and Hall *(10)* have also developed a thermocured bacon involving an antioxidant treatment previous to curing the bacon in a brine solution at a temperature of 127-130° F. (52.8-54.4° C.). This bacon is cured in a 12-hour period and then smoked as in other methods. The procedure is covered by a U. S. patent *(11)*.

Preliminary tests with 6 bellies were made in a 60° salometer pickle at 120° F. (48.9° C.) for 24 hours. These were cured to check visually the keeping quality, completeness of cure, and general appearance. In palatability they compared very favorably with 21-day, dry, box-cured bacon from the meat laboratory and one of the top brands of commercial bacon.

METHODS AND MATERIALS

The main experiment consisted of 4 groups, each group containing green bellies from 6 hogs, which were cut to afford 4 treatments. The cuts from front and rear were rotated as equally as possible. These 4 groups were designed to determine the optimum strength of pickle, temperature, and time for an accelerated cure.

The strength of brine was varied in Groups 1 and 2 to determine a satisfactory pickle. Group 1 consisted of 4 treatments as follows: dry cure, 21 days at 38° F. (3.3° C.); 55° pickle, 24 hours at 120° F. (48.9° C.); 65° pickle, 24 hours at 120° F. (48.9° C.); and 75° pickle, 24 hours at 120° F. (48.9° C.). Group 2, a continuation of Group 1, was as follows: dry cure, 21 days at 38° F. (3.3° C.); 75° pickle, 24 hours at 120° F. (48.9° C.); 85° pickle, 24 hours 120° F. (48.9° C.); and 95° pickle, 24 hours at 120° F. (48.9° C.).

Group 3, set to determine the optimum temperature, had the following treatments: dry cure, 21 days at 38° F. (3.3° C.); 55° pickle, 24 hours at 115° F. (46.1° C.); 55° pickle, 24 hours at 120° F. (48.9° C.); and 55° pickle, 24 hours at 125° F. (51.7° C.).

Group 4, devised to establish the optimum time in cure had the following treatments: dry cure, 21 days at 38° F. (3.3° C.); 55° pickle, 18 hours at 120° F. (48.9° C.); 55° pickle, 24 hours at 120° F. (48.9° C.); and 55° pickle, 30 hours at 120° F. (48.9° C.).

The green bellies were in the 10-12-pound weight class from Hampshire hogs weighing from 200 to 230 pounds. The hogs were slaughtered, chilled, and cut at the University of Illinois meat laboratory.

The ingredients used in all of the curing solutions and mixtures consisted of bulk finely granulated salt (sodium chloride), finely granulated pure cane sugar (sucrose), Merck's C. P. sodium nitrite, and Merck's C. P. sodium nitrate. Care was taken to see that the curing ingredients came from the same production lot.

The rack used to hold the meat on edge in the curing vat was constructed of ½ inch mesh hardware cloth. The curing vat was a stainless steel "No Air Wax" dipping vat which had an electric heating unit with automatic temperature control. The curing boxes utilized for the dry-cure method were typical steel-lined dry-cured boxes (10 inches x 20 inches x 30 inches) such as are used by a locker plant. A Griffith electrically heated, automatically controlled portable smokehouse was used for the main experiment. An old farm-type brick smokehouse, in which heat for smoking is furnished by a wood fire, was employed for the preliminary curing trials. Griffith salometers adjusted to 60° F. (15.6° C.) were used in determining the degree of pickle. The General Electric Recording Spectrophotometer, for the visible region of the spectrum (400-700 $m\mu$), was employed to measure the percentage of light reflected by the lean and by the fat of the sliced bacon. A Coleman Spectrophotometer, Model 14, was utilized to obtain color transmittance in the nitrite and nitrate determinations. A Beckman pH meter was used to determine the pH of the pickle solutions.

[a] The data are taken from a thesis submitted by Verlin K. Johnson in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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The hogs were slaughtered in the morning and chilled 24 to 30 hours before being cut. Except in Group 1, when lack of experience caused delay, the box-cure treatment and the first of the other three treatments were started the following evening. This made it possible to put the third treatment of the accelerated method into cure before it had been in the cooler 4 days.

The box cure of each group was handled in the following manner. The 6 pieces of green bellies for the test were weighed and then packed with bellies from other hogs in order to get a normal treatment. The amount of meat in each group is shown in Table 1.

The formula for the dry cure consisted of 3 pounds of salt (sodium chloride), 1¼ pounds of sugar (sucrose), 85 g. of sodium nitrate, and 28 g. of sodium nitrite per 100 pounds of fresh meat. The amounts of curing ingredients used in each group are shown in Table 1. The formula was thoroughly mixed and rubbed on the green bellies until they were covered. The bellies were packed in the curing box with skin side down, except for the last one which was placed in the box skin side up. The box was covered with a weighted lid. The box of bacon was overhauled on the seventh and fourteenth days, reversing the order of the bellies. At 21 days the bellies were removed from the curing box, wiped dry, weighed, and soaked for 90 minutes in water at 80-90° F. (26.7-32.2° C.). The bellies then were hung on bacon hangers, allowed to drain until dry, and

TABLE 1

Weights of meat and curing ingredients used in box cure

Group	Total meat	Experimental meat	Sodium chloride	Sugar	Sodium nitrate	Sodium nitrite
	pounds	*pounds*	*pounds*	*pounds*	*g.*	*g.*
1	129.0	33.95	3.87	2.26	109.7	36.10
2	108.0	34.66	3.24	1.89	91.8	30.24
3	111.6	34.60	3.36	1.96	95.2	31.40
4	113.2	33.20	3.39	1.98	96.0	31.60

hung in the smokehouse. The smokehouse was preheated to 135° F. (57.2° C.) and it was left at that temperature with the ventilators open until the internal temperature of the bellies reached 100° F. (37.8° C.). At this time the smoke generator was started and the temperature reduced to 120° F. (48.9° C.). The heat and smoke were kept on for a period of 12 to 14 hours. If the bellies had not absorbed sufficient smoke by this time, as determined by visual inspection, the heat was shut off and the smoke generator was kept in operation. Every effort was made to keep treatment in the smokehouse uniform for all methods of cure. The bacon was cooled in the smokehouse to room temperature and then moved into the cooler. The finished bacon was weighed after the internal temperature had reached 38° F. (3.3° C.).

The degree of pickle of all brines was determined before the sodium nitrate, sodium nitrite, and sugar were added.

The curing solutions were made from tap water [approximately 60° F. (15.6° C.)] so that major corrections would not need to be applied to the salometer reading. The weights of meat, curing ingredients, salometer readings of pickle (before and after the addition of sodium nitrate and nitrite), times, and temperatures are shown in Table 2.

The curing solutions were brought to the desired temperatures before the meat was submerged in the vat. The time in cure and the temperature schedules shown in Table 2 were observed rigidly with only one exception. In the 85° pickle treatment of Group 2 a short circuit occurred, causing the heat to drop from 120° F. (48.9° C.) to 110° F. (43.3° C.) so 30 minutes were added to the time in cure.

When the bellies were removed from the cure they were cooled to 38° F. (3.3° C.) before being weighed. The cured bellies were soaked and smoked in the same manner as those from the box cure.

Within 2 weeks after smoking, each cut from each treatment was checked for light reflection on the spectrophotometer. Samples of fat and lean were taken from a ⅜-inch slice of bacon which was removed from the cut at least 1½ inches from the end. These samples were cubical and taken from as near the

TABLE 2

Accelerated curing of bacon of groups 1-4[a]

	Salometer, initial[a]	Salometer, Final[a]	Meat	Water	Salt	Time	Temperature
	°	°	*pounds*	*pounds*	*pounds*	*hours*	*° C.*
Group 1	55	59	33.50	155	27.37	24	48.9
	65	69	33.16	160	33.66	24	48.9
	75	80	33.43	156	38.89	24	48.9
Group 2	75	82	34.63	156	39.11	24	48.9
	85	93	35.57	146	43.55	24	48.9
	95	99	34.96	146	49.00	24	48.9
Group 3	55	60	35.04	156	27.00	24	46.1
	55	61	35.08	156	27.00	24	48.9
	55	60	33.38	156	27.00	24	51.7
Group 4	55	60	32.89	156	27.00	18	48.9
	55	60	33.00	156	27.00	24	48.9
	55	60	32.33	156	27.00	30	48.9

[a] Each curing solution contained 5 pounds of sugar, 70 g. of sodium nitrite, and 20 g. of sodium nitrate.

center of the slice as possible in order to get the proper size samples. This size was used because an aperture 3/16 by 5/16 inches was best suited for mounting material such as bacon fat on the spectrophotometer. The recordings of the percentage of light reflection were corrected for the aperture used, and the mean light reflection of the fat and lean was calculated according to Bouma *(2)*.

Palatability tests were made comparing the 4 treatments of each group. The palatability committee for the experimental tests, Groups 1, 2, 3, and 4, consisted of 10 selected faculty members from the Animal Science and Home Economics Departments. The palatability tests were conducted in the Home Economics Foods Laboratory. In each of these tests the sample was taken from the same hog. The bacon slices used were taken 1½ inches from the center of each side. The slices were cut about 9/64 inch thick on a mechanical slicer. Then ½ inch was removed from each end of bacon slice to insure more uniformity in cooking. The samples were cooked on racks in shallow (¾-inch) bacon-cooking pans in an oven at 450° F. (232.2° C.). The palatability score card had 7 possible ratings (1-7) for the following: color, aroma, flavor, salt, and texture. Of these 7 ratings, 1 was the most desirable.

A sample from each cut was saved for chemical analyses, and these were wrapped in aluminum foil and frozen. These samples were separated into fat and lean, and the weight of each was recorded. After separation of the fat and lean, the samples were ground, thoroughly mixed, and frozen for chemical analyses.

The sodium chloride in the ground lean and fat from each of the 96 cuts was determined by a method described by Davies *(6)*.

The sodium nitrite of the lean was determined by the sulfanilic acid method *(1)*. This method was modified so that a Coleman spectrophotometer could be used in comparing colors. Common pyrex test tubes were used instead of 100 ml. Nessler tubes. The amount of unknown was made to 10 ml., rather than 100 ml., and 1/10 as much reagent as described was used for color development. The percent of transmittance was read on the spectrophotometer in calibrated cuvettes. The color readings were compared with a standard curve established at the beginning of the determination. The standard curve was checked periodically.

The meta-xylenol method *(1)* with adaptations for spectrophotometer readings, was used for the determination of the sodium nitrate in the lean. In place of comparing the color development by human vision, as described, knowns were determined and a standard curve prepared from the transmittance readings on the Coleman spectrophotometer. The unknowns were determined and a standard curve prepared from the transmittance readings on the Coleman spectrophotometer. The unknowns were compared to the standard curve, and amounts of sodium nitrate were determined.

Nitrite and nitrate determinations were made of the fat, but the values obtained were too low to read accurately on the standard curve, so these determinations were abandoned.

All chemical determinations were run in triplicate. When checks were not obtained, the determinations were repeated until checks were obtained.

The shrink data and that from the color studies were tested for significance by use of the *t* test for paired differences *(13)*. In all other cases the significance of difference was measured by the analysis of variance *(12)*. A *p* value of 0.01 was considered highly significant (odds of 99 to 1 or greater); one of 0.05 was considered significant (odds of 19 to 1). The exact formulas used were developed by Professor H. W. Bean, Department of Animal Science, University of Illinois.

RESULTS AND DISCUSSION

The color of meat is highly important to the consumer and most meat is selected on the basis of appearance. In bacon, especially, color plays an important role in consumer acceptance. For the most part the consumer selects the bacon that is the most attractive. Brightness of the lean is closely related to the attractiveness of sliced bacon. Hence, the brightness of the bacon produced from each treatment in this experiment was measured on the spectrophotometer.

The amount of light reflected was measured in percentage on the General Electric Recording Spectrophotometer on the basis of plates smoked with MgO as 100%. The absolute reflectance of MgO is approximately 98%. However, nothing has, as yet, been found more suitable than freshly prepared magnesium oxide for a fundamental standard of spectral directional reflectance. For these measurements the standard plates were smoked freshly each time the spectrophotometer was used.

In each group the bacon from the dry-box cure (21 days) was compared with bacon from the quick-cure treatments.

Table 3 gives a summary of the spectrophotometer color studies of the lean. In Group 1, bacon from the dry-box cure (21 days) was compared with bacon from the 55°, 65°, and 75° pickle, all cured at 120° F. (48.9° C.) for 24 hours. The 55° and 65° pickle resulted in leans which gave percentages of light reflected that were 5.77% and 4.23%, respectively, greater by difference than that of the 21-day cure. These differences were highly significant. On a percentage basis, the values may be exrpessed as being 49 and 36% greater, respectively, than the values found for the dry cure. The difference between the light reflectance of the lean from the bacon cured in 75° pickle and that from the 21-day cure was not significant. This may have been due to the fact that it was not put in cure until it had been in the cooler 5½ days after having been cut.

In Group 2 the reflectance of light by the lean was greater in all three quick-cure samples than in the 21-day dry cure. The magnitude of this difference was significant in the pairing of the 75° pickle and the dry cure and highly significant in the other two comparisons.

Bacon from the 115°F. (46.1° C.) 55° pickle, quick-cure of Group 3, where temperature was the only variable, had a greater light reflectance value than the dry cure, but this difference was not significant. The bacon from the 120° and 125° F. (48.9° and 51.7° C.) treatments also showed a greater light reflectance than did the bacon from the dry cure. These differences were significant.

TABLE 3

Variations in light reflectance, shrinkage, sodium chloride, and sodium nitrite values with time, temperature, and degree of pickle

Group 1	Dry cure 21 days 38° F.	55° pickle 24 hours 120° F.	65° pickle 24 hours 120° F.	75° pickle 24 hours 120° F.
Mean light reflected, %	11.65	17.42	15.87	13.95
Mean NaCl in lean, %	4.22	4.74	5.09	5.15
Mean $NaNO_2$ in lean, p.p.m.	74.43	26.23	29.85	25.82
Mean shrinkage out of smoke, %	8.47	11.52	11.60	11.44
Group 2	**Dry cure 21 days 38° F.**	**75° pickle 24 hours 120° F.**	**85° pickle 24 hours 120° F.**	**95° pickle 24 hours 120° F.**
Mean light reflected, %	11.32	17.04	16.88	17.64
Mean NaCl in lean, %	4.59	5.83	5.69	6.11
Mean $NaNO_2$ in lean, p.p.m.	123.92	23.76	25.03	20.07
Mean shrinkage out of smoke, %	8.94	10.84	9.81	10.77
Group 3	**Dry cure 21 days 38° F.**	**55° pickle 24 hours 115° F.**	**55° pickle 24 hours 120° F.**	**55° pickle 24 hours 125° F.**
Mean light reflected, %	12.44	16.83	19.21	20.00
Mean NaCl in lean, %	5.33	5.06	5.03	5.02
Mean $NaNO_2$ in lean, p.p.m.	174.08	28.93	27.98	29.58
Mean shrinkage out of smoke, %	7.43	4.98	12.02	12.12
Group 4	**Dry cure 21 days 38° F.**	**55° pickle 18 hours 120° F.**	**55° pickle 24 hours 120° F.**	**55° pickle 30 hours 120° F.**
Mean light reflected, %	11.39	17.49	18.56	19.97
Mean NaCl in lean, %	5.56	3.96	4.24	4.41
Mean $NaNO_2$ in lean, p.p.m.	255.97	21.61	31.16	28.36
Mean shrinkage out of smoke, %	10.21	11.38	12.07	11.55

In Group 4, where time in cure was the only variable, the bacon from the 18- and 24-hour cures had 6.09 and 7.16% greater light reflectance values, respectively, than did the box cure. These differences were significant. The lean from the 30-hour cure had 8.58% greater light reflectance than did that from the box-cure when expressed by difference. This distinction was highly significant. On a percentage basis this difference was 75% greater than the reading from the box-cure bacon.

These color measurements showed that the quick-cured bacon had a brighter lean than did that of the dry-cured bacon. This also was shown by color photographs. The color photographs also showed that the color of lean was darker from the box-cure than from the quick-cure methods.

The fat of the quick-cured bacon was as bright as that from the dry-cured bacon.

The palatability tests reflected the greater percentage of sodium chloride in the lean found in the cuts cured in the 75°, 85°, and 95° pickles. The aroma factor had the greatest variation in the 4 groups. This variation was not in the same direction and occurred only in three groups. The aroma variation may be due to the variations in smoke which occurred between the dry-cure treatments and the quick-cure treatments. Most of the differences that appeared in the results of the palatability test were small and not consistent. In general, it seemed evident that the bacon produced from the dry cure method and that from the 55° pickle, 24 hours, 120° F. (48.9° C.) treatment were equally acceptable to the palatability committee.

The percentage of sodium chloride found in the lean of all groups is summarized in Table 3. The degree of pickle used in the quick-cure treatments affected the amount of sodium chloride in the bacon lean. In general, the higher the degree of pickle, the greater was the amount of sodium chloride in the lean. The amount of sodium chloride in the lean from the lower degrees of pickle (55° and 65°) did not differ greatly from that of the dry cure. When the degree of pickle and time were constant, the temperatures tested had no significant effect upon the amount of sodium chloride in the lean. None of the treatments had any appreciable effect upon the amount of sodium chloride in the fat. The average sodium chloride in the fat from Groups 1, 2, 3, and 4 was 0.63, 0.69, 0.73, and 0.72% respectively. From this it was evident that the amount of sodium chloride found in the bacon (fat and lean) was directly related to the amount of lean in the sample.

In Groups 1 and 2 the degree of pickle did not have any significant effect upon the amount of sodium nitrite (expressed in p.p.m.) absorbed by the bacon of the quick-cure treatments. Neither did the temperatures tested in Group 3 have any effect upon the amount of sodium nitrite absorbed by the quick-cure treatment of the group. Also, the variation in time tested in Group 4 caused no significant difference in the amount of sodium nitrite taken up by the quick-cure treatments. A summary of the sodium nitrite found in the lean is shown in Table 3. The mean value of sodium nitrite present in all the quick-cure treatments of all four groups was 26.53 p.p.m. The mean values in p.p.m. of sodium nitrite present in the bacon from the quick cures of each group were as follows: Group 1, 27.30; Group 2, 22.95; Group 3, 27.04; and Group 4, 28.83. The amount of sodium nitrite in the lean of the bacon from quick-cure was much lower than the amount allowed by the U. S. Department of Agriculture in government inspection, less than 200 p.p.m. From both the color measurements and the appearance of the bacon it was evident that there was sufficient sodium nitrite present to establish good color. This is in keeping with the statement *(3)* that an amount of sodium nitrite of 10 p.p.m. is sufficient for good color development. The mean value of sodium nitrite present in the bacon from the box cure was 157.10 p.p.m. In all 4 groups the sodium nitrite, measured in p.p.m., was several times greater in the lean from the dry cure than in that from the quick-cure. When these differences were tested by the analysis of variance they were highly significant. The differences in sodium nitrite in the lean from the quick-cure treatments within each group were not significant. The amount of sodium nitrite absorbed by the fat was very minute under all conditions.

In Group 1 there was several times more sodium nitrate in the lean from the dry-cure bacon with 165.10 p.p.m. than in that from any of the 3 quick-cure treatments which had 32.00, 21.81, and 14.83 p.p.m., respectively. The difference was highly significant when tested by the analysis of variance. The differences between mean values for the bacon from the 3 quick-cure treatments were not significant. The mean values of sodium nitrate in the lean of the quick-cure treatments of Group 2 averaging 22.95 p.p.m. appeared to be different from 123.92 p.p.m. of the dry-cured bacon. However, the analysis of variance did not show a significant difference. In Group 3 the differences in the amounts of sodium nitrate in the lean between the quick-cure treatments and the dry-cured bacon were not significant. Group 4 disclosed a picture similar to Group 1. The lean of the dry-cured bacon had 106.61 p.p.m. of sodium nitrate, while bacon lean from the three quick-cure treatments had a mean value of 26.87 p.p.m. The analysis of variance showed that this difference was highly significant. The differences in amount of sodium nitrate in the bacon from the three quick-cure treatments were not significant. The sodium nitrate in the fat, like the sodium nitrite, was so minute that it was not possible to obtain accurate determinations. This was logical, since the percentage of water-soluble material in fat is very low.

The shrinkage (on basis of initial weight) in the quick-cure (55°, 65°, 75° pickle of Group 1 were 2.96, 3.14, and 2.98% greater, respectively, than was that of the box-cure as shown in Table 3. When analyzed by the paired difference method, these were highly significant differences. The differences in percentage of shrink between the quick-cure methods were not significant. In Group 3, the quick-cure treatments 115° F., 120° F., and 125° F. (46.1° C., 48.9° C., and 51.7° C.) resulted in 4.58, 4.59, and 4.69% greater shrinkage, respectively, than did that of the box-cure. These differences were all highly significant. In Group 4, the percentage of shrink from the box-cure was 1.87% lower than was that of the bacon from the 24-hour cure. This difference was significant. When shrinkage from the 18- and 30-hour cures were compared to the box cure, the differences were not significant. In 12 comparisons of the shrinkages of the quick-cured bacon with the respective box-cured bacon, all had a higher shrink than did the box-cured bacon. In 6 of the 12 comparisons, the differences were highly significant; in 2 cases the differences were significant; while in 4 cases the difference was not significant, although the shrinkage was higher in the quick-cured bacon.

The shrinkage data reported here does not coincide with the 95% yield reported in the thermocuring process *(10, 11)*. However, the shrinkage in this experiment probably could have been reduced had the project been executed with sufficient equipment to carry on the three quick-cure treatments simultaneously. This would have prevented the cooling of the bacons following the curing period which no doubt caused some extra dehydration.

The pH of the quick-cure solutions was determined at the beginning of cure, 12 to 14 hours after the start of cure, and at the completion of cure. The average pH of the pickles at the beginning of the curing period was 7.40. At a point midway in the quick-curing period the mean pH was 7.15. At the completion of the curing period, the mean pH was 7.20. The pH readings were in general agreement with the statement that the pump pickles used in Canada are usually around 7.8 *(5)*. Studies of the pH of curing with sterilized pork in sterile

solutions *(7)* resulted in the conclusion that for optimum color fixation a pH of 5.2 to 6.0 was desirable. According to another investigator *(9)* a very narrow pH range (6.4-7.0) is necessary for the development of bacteria and flavor-producing enzymes. According to the theory that bacteria are not needed to develop flavor and color, it seems possible that the narrow pH range (5.2-6.0 or 6.4-7.0) may not be necessary. The color fixation resulting from the quick-cures of this experiment was very good, as shown in the discussion of the color measurements; yet, the pH of the pickle was above 7.1 at all times. The palatability tests also demonstrated that flavor was not impaired by the slightly alkaline pH.

SUMMARY

The main experiment was designed to determine the optimum strength of pickle, temperature, and time for an accelerated cure for bacon. The strength of pickle was varied in Groups 1 and 2. Group 1 consisted of 4 treatments as follows: dry-cure, 21 days at 38° F. (3.3° C.); and 55° pickle, 24 hours at 120° F. (48.9° C.); 65° pickle, 24 hours at 120° F. (48.9° C.); and 75° pickle, 24 hours at 120° F. (48.9° C.). Group 2 was as follows: dry cure, 21 days at 38° F. (3.3° C.); 75° pickle, 24 hours at 120° F. (48.9° C.); 85° pickle, 24 hours at 120° F. (48.9° C.); and 95° pickle, 24 hours at 120° F. (48.9° C.). The results of the palatability test of the bacon from the 6 quick-cured treatments of Groups 1 and 2 showed that the bacon from the 55° and 65° pickle were very similar and the most acceptable of the 6 quick-cure treatments. Of these two treatments, the 55° pickle was chosen as the most desirable, since it produced bacon with sufficient salt and required less salt in preparation of the pickle solution.

Group 3, set up to determine the optimum temperature, had the following treatments: dry-cure, 21 days at 38° F. (3.3° C.); 55° pickle, 24 hours at 115° F. (46.1° C.); 55° pickle, 24 hours at 120° F. (48.9° C.); and 55° pickle, 24 hours at 125° F. (51.7° C.). The differences between the bacon from the three quick-cures of this group were very small. The shrinkage out of smoke, based on the green or initial weight, was somewhat greater in the 125° F. (51.7° C.) treatment. The 120° F. (48.9° C.) temperature was considered optimum.

Group 4, devised to establish an optimum time, had the following treatments: dry-cure, 21 days at 38° F. (3.3° C.); 55° pickle, 18 hours at 120° F. (48.9° C.); 55° pickle, 24 hours at 120° F. (48.9° C.); and 55° pickle, 30 hours at 120° F. (48.9° C.). The data from the bacon of the three quick-cure treatments of this group were highly similar. The most usable treatment of three is the 24-hour treatment because of its greater adaptability to commercial use.

The color measurements on the spectrophotometer showed that the quick-cured bacon had a brighter lean than that of dry-cured bacon. This also was borne out by the color photographs. The color photographs, likewise, showed that the lean of the dry-cured bacon was darker than that from the quick-cured bacon.

The palatability tests reflected the greater percentage of sodium chloride in the lean found in the cuts which were cured in the 75°, 85°, and 95° pickles. Other differences checked by the palatability test were very small and not consistent. The palatability of the bacon produced by the 55° pickle, 24 hours at 120° F. (48.9° C.) treatment was equal to that of the dry-cured bacon.

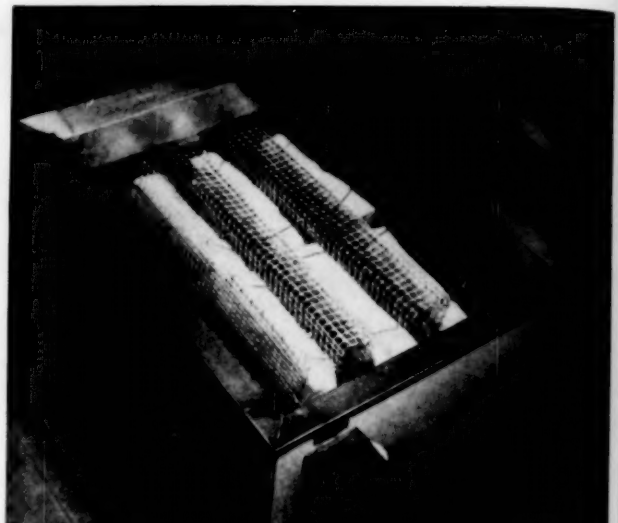

Figure 1. The curing vat used showing vat with rack and green bellies submerged in the pickle.

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The Fusel Oil Content of California Wines[a]

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The fusel oil content of 367 California commercial wines entered at the 1947 California State Fair was determined. Included were samples of nearly all of the commercial wine types. Consideration is given to the significance of the fusel oil content in the organoleptic character of wines and the importance of fusel oil in wines to be distilled is pointed out.

Fusel oil from the industrial standpoint refers to the water-insoluble fraction which accumulates in a middle region of a fractionating column during the distillation of yeast-fermented liquids. This oil fraction may be continuously withdrawn from a distilling unit and separated by washing and decantation. It is largely made up by alcohols of molecular weights higher than ethanol, especially butyl and amyl isomers, but it also includes small percentages of several other alcohols, acids, esters, and other substances.

In a narrower sense, the term fusel oil is used synonomously with the term higher alcohols, since methods for analytically measuring amounts in solutions are based primarily upon properties of the alcohols. This is the sense in which the term "fusel oil" is used in this report.

The nature, origin, and occurrence of higher alcohols in alcoholic beverages has interested many workers. Various authors including von der Heide and Schmitthenner *(6)* and Genevois *(4)* have reviewed the information. Some early investigators sought to attribute the origin to the carbohydrate, others to fats in the substrate fermented by yeasts. Ehrlich in 1906 *(3)* demonstrated that certain amino acids when utilized by yeast are the precursors of the alcohols which constitute fusel oil.

Neubaurer and Fromherz *(7)* presented evidence to indicate that the conversion by yeasts of amino acids to alcohol residues proceeds as follows: a, deamination of the amino acid with formation of an alpha ketonic acid; b, decarboxylation of the ketonic acid to an aldehyde of one less carbon atoms; and c, reduction of the aldehyde to an alcohol. It is now generally accepted that the amino acids are broken down to furnish nitrogen for the cellular growth of yeast. However, the production of higher alcohols during fermentation requires the simultaneous conversion of sugars to ethyl alcohol by living yeasts *(5)* and the appearance of the alcohols in the medium coincides with the formation of ethanol rather than with the phase of primary cell reproduction *(1)*.

Investigations of fusel oil in alcoholic beverages have been principally centered in distilled liquors. The influence of fusel oil contents upon the organoleptic character of brandies, whiskies, rums, etc., is generally recognized. A determination of higher alcohols in these products is regularly a part of the usual laboratory analysis and considerable data are available in published literature. Abnormal concentrations of fusel oil can be readily detected by organoleptic tests.

In undistilled alcoholic beverages, such as wines, the higher alcohols, constituting a relatively lesser quantity of the total substances contained, have less though undoubtedly not an insignificant influence upon their organoleptic character. Knowledge of the fusel oil contents of wines of different types or from different regions or of recognized quality levels might prove a useful criterion in estimating their organoleptic character, and their suitability for distilling wines.

Information on the higher alcohol contents of wines is very meager. Windisch *(10)* in 1906 found the fusel oil content of 14 German wines to be 0.013 to 0.055 volume percent, averaging 0.028. Calculated as mg. per 100 ml., assuming the density of fusel oil as 0.81 g. per ml., the range would be 16 to 68, averaging 35. Pettigiani *(9)* reported 15 to 30 mg. of higher alcohols per 100 ml. for 24 Argentinian wines. In 12 white wines of Italy, Cioffi *(2)* reported a considerably higher average of 0.071 ml. of higher alcohol per 100 ml. of wine, equivalent to 87 mg. He also found 0.062 ml. or 77 mg. per 100 ml. for 22 Italian red wines. Apparently no data for California wines have been reported before.

The fusel oil contents of 367 California commercial wines were determined. All of these wines were entered for judging at the 1947 California State Fair. An original unopened bottle of each was brought to Davis and stored at 55° F. (13° C.) until opened for analyses about a year later. Almost all of the commercial California wine types are included in the results. However, there are no analyses for the class, white port, and only a small number for sherry. Altogether, there were 438 wines at the competition.

EXPERIMENTAL

Method of analysis. A colorimetric method based on the Komarowski reaction and a modification of the Penniman *(8)* procedure was used. This depends upon the color formed by the interaction of higher alcohols with a cyclic aldehyde in concentrated sulfuric acid. The details of the procedure used are as follows:

Place 50 ml. of wine and about 15 ml. of water in a 300 ml. Kjeldahl flask and distil over about 50 ml. Transfer the distillate,[b], using 15 ml. of water for rinsing, to a 250 ml. Erlenmeyer flask having a standard taper ground glass joint. Add 0.25 g. of silver sulfate, 0.5 ml. of 18 *N*. sulfuric acid (1 + 1),

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[b] Usually a 100 or 200 ml. volume of the wine is distilled for the purpose of determining the total or ethyl alcohol in the distillate by a densimetric method. It is then convenient to start with 50 ml. of this distillate, thereby eliminating the first distillation step.

a few Cargill boiling stones, connect to a water-cooled condenser,[c] and reflux for 15 minutes. Add 5 ml. of 25% sodium hydroxide solution to make alkaline and a small pinch of granular zinc to prevent bumping and reflux for 30 minutes more. The purpose of this treatment, besides neutralizing acids and saponifying esters, is to oxidize aldehydes. In this latter, it is only partially effective. Distil about 50 ml. and make to volume at standard temperature.

Carefully pipet 1 ml. of the purified distillate into a 25 × 150 mm. Pyrex rimmed test tube and set in an ice bath. Add from a free flowing pipet 20 ml. of an ice-cold concentrated sulfuric acid into which has been dissolved *p*-dimethylaminobenzaldehyde at the rate of 0.5 g. per liter, swirling the tube in an ice cold bath during the addition. Some 12 to 15 tubes may be prepared at one time. Cap each tube by pressing on aluminum foil and place in a vigorously boiling water bath for 20 minutes. After about a minute of heating each tube should be momentarily lifted out of the bath and again swirled to insure complete mixing. After the heating period, transfer to an ice bath for several minutes. Then bring to room temperature and measure the color intensity in a filter photometer or spectrophotometer, using the solvent as reference liquid. Obtain the content of fusel oil from a transmittance curve made with standard fusel oil solutions and preferably prepared in the same batch as the samples to be read. The standard solutions were made from a weighed quantity of a mixture of redistilled C. P. alcohols, 4 parts by volume of isoamyl alcohol and 1 part of isobutyl alcohol.

The color measurements were made with a Coleman Model 11 spectrophotometer set at 480 $m\mu$ and with 8 × 8 mm. square cuvettes.[d]

RESULTS

Fusel oil content of table wines. The fusel oil or higher alcohol content found for the several classes of white table wines, totaling 120 samples, are given in Table 1. The values expressed on the basis of the ethyl alcohol present were calculated by dividing the measured contents of higher alcohols by the volume percent of total alcohols in each wine.[b] The mean values for each type of wine were very consistent, differing but little from the overall average of 25 mg. of fusel oil per 100 ml. of wine or 208 mg. per 100 ml. of ethyl alcohol for all the white wines. It had been anticipated that significant differences might be found between some classes, especially those in which the wines are derived from specific grape varieties or closely related ones. However, with the possible exception of the sweet sauterne class, significant variations are not apparent from the data. Standard deviation of fusel oil values were calculated for each wine type to indicate the dispersion of the data.

Table 2 contains corresponding results found for 130 red table wines. The 11 rosé and 5 pink sparkling wines are included, although these are not strictly red types. Actually the mean fusel oil values for these two types were the two lowest of those listed in Table 2. As in the case of the white wines, the differences between the classes of red wines are seldom more than the standard deviation. Considering all 130 red wines, the average content of higher alcohols was 28.7 mg. per 100 ml. of wine or 235 mg. per 100 ml. of ethyl alcohol, about 10% more than that of the 120 white wines. This is contrary to the findings of Cioffi referred to earlier.

Fusel oil content of dessert wines. The production of dessert wines, including the appetizer or sherry types, involves the addition of neutral or fortifying brandy to a partially fermented must or wine. The final alcohol content is about 20 volume percent whereas in the table wines it is about 12%. From annual production records of California wines for the four-year period, 1946-49, it was calculated that the actual volume of brandy added constituted an average of 15.9% of the volume of all dessert wines produced. On an alcohol basis, this means that almost 75% of the alcohol in the average dessert wine was added through the medium of the brandy, or only 25% arose directly from fermentation. Since the opportunity exists for eliminating a major portion of the fusel oil from the neutral brandy during its distillation, one might expect to find low fusel oil contents in dessert wines.

[c] The use of a special combination apparatus, Ace Glass Co., Drawing M-1022, for refluxing and subsequent distilling is very convenient.

[d] More recently a wavelength setting of 520 $m\mu$ and 13 × 13 mm. cuvettes have been adopted as standard.

[b] The total or ethyl alcohol content was obtained by distillation and use of a standard hydrometer, reading in volume percent of ethyl alcohol.

TABLE 1
Fusel oil content of white table wines

Type	Number of samples	Fusel oil in mg.							
		Per 100 ml. of wine				Per 100 ml. of ethyl alcohol			
		Minimum	Maximum	Mean	S.D.[g]	Minimum	Maximum	Mean	S.D.
Chablis	14	21.0	30.7	25.3	2.7	183	282	219	32
Folle blanche	2	23.8	30.9	27.4	3.9	209	253	231	31
Moselle	4	22.2	28.6	25.7	2.7	205	255	226	22
Rhine	8	18.2	32.4	26.7	4.6	141	277	217	45
Riesling	17	21.6	34.2	26.6	3.7	175	297	226	41
Sauterne	27	16.7	32.4	24.3	4.0	142	265	201	32
Sweet Sauterne	10	16.2	29.1	21.8	3.5	131	220	175	23
Sauvignon blanc	2	28.5	30.6	29.0	1.8	225	279	252	38
Semillon	5	22.4	32.0	25.3	3.9	160	260	197	38
Sweet Semillon	1	.....	.....	18.8	.....	.....	.....	182	.....
Traminer	5	22.3	36.6	28.5	5.4	186	308	232	46
Miscellaneous	7	18.9	25.2	22.2	2.2	158	196	183	14
White Pinot[e]	8	22.8	31.8	26.1	3.1	181	248	210	25
Sparkling[f]	10	18.0	31.8	24.6	4.8	167	256	202	34
Total	120	16.2	36.6	25.0	3.5	131	308	208	36

[e] Includes Pinot blanc and Chardonnay.
[f] Includes 8 California champagne, 1 Sparkling Muscat, and 1 Carbonated Moselle.
[g] S.D.: Standard deviation.

TABLE 2
Fusel oil content of red table wines

Type	Number of samples	Fusel oil in mg.							
		Per 100 ml. of wine				Per 100 ml. of ethyl alcohol			
		Minimum	Maximum	Mean	S.D.	Minimum	Maximum	Mean	S.D.
Barbera	4	24.3	32.4	27.8	3.7	199	238	222	19
Burgundy	23	19.5	34.5	28.1	4.0	146	286	229	33
Cabernet	13	25.2	38.7	33.6	3.8	198	297	263	30
Claret	25	19.7	41.6	28.3	5.1	156	344	234	46
Chianti	4	27.0	37.8	30.1	5.2	200	295	236	41
Charbono	2	32.8	34.7	33.8	1.3	262	286	274	17
Pinot noir	9	23.4	33.3	28.8	3.9	185	286	231	37
Pinot, red	2	29.4	38.4	33.9	6.4	256	300	278	31
Rosé	11	16.0	33.9	24.9	5.4	142	286	215	51
Zinfandel	20	21.3	41.7	31.6	4.2	162	298	250	37
Miscellaneous	3	20.7	31.5	26.6	5.5	167	250	216	49
Sparkling Burgundy	9	14.0	31.5	25.7	5.4	189	281	221	30
Pink Sparkling	5	16.2	24.9	21.9	3.5	175	219	200	20
Total	130	14.0	41.7	28.7	5.3	142	344	235	40

TABLE 3
Fusel oil content of dessert wines

Type	Number of samples	Fusel oil in mg.							
		Per 100 ml. of wine				Per 100 ml. of ethyl alcohol			
		Minimum	Maximum	Mean	S.D.	Minimum	Maximum	Mean	S.D.
Angelica	10	24.3	90.0	40.9	20.7	120	486	208	108
Madeira	2	29.1	33.8	31.5	3.2	154	173	164	13
Muscatel	28	15.6	48.9	35.6	9.8	78	253	221	84
Port	28	22.5	53.8	38.4	10.8	113	291	197	54
Sherry	4	34.5	44.7	40.1	4.5	176	212	197	14
Sherry, dry	5	18.8	39.6	29.2	8.6	93	201	148	45
Tokay	18	18.3	48.1	25.5	7.5	93	250	187	78
Vermouth, dry	12	31.8	49.1	38.8	4.5	170	268	209	28
Vermouth, sweet	10	21.6	57.3	36.9	4.0	114	315	212	71
Total	117	15.6	90.0	37.4	10.4	78	486	202	74

The results for 117 wines among 9 dessert classes are indicated in Table 3. Actually, the higher alcohol contents were relatively low for many individual wines, but were also quite high in others, with a resulting wide range of values. The mean value of 202 mg. per 100 ml. of ethyl alcohol is only a little less than for the table wines, suggesting that the distilleries were eliminating from the brandy only a small portion of the fusel oil present in the wine distilled. Therefore, the standard deviations of fusel oil contents for the dessert wines were comparatively large, attributable in the main to varying contents of fusel oil in the fortifying brandy which had been used.

This is indicated from the data of Table 4 in which the higher alcohols are given for 3 wines entered by each of 4 wineries. Based on personal observation of the writer, the first two wineries employed practices of successfully eliminating fusel oil in the distillation of their neutral brandy while the last two did not. The fusel oil content of their wines reflects this.

However, other variables in wine-making procedures could influence the quantities of higher alcohols. Sherry and tokay wines, for example, contain less sugar than other types, so that, in comparison, a larger portion of the ethyl alcohol content is derived directly from fermentation rather than from the fortifying brandy. The same would be true for types containing more sugar, such as port, if, as is sometimes done, the wine maker allowed the fermentation to proceed until most of the sugar was fermented and converted to alcohol and then added grape concentrate to provide the necessary sweetness.

TABLE 4
Fusel oil content of dessert wines from certain wineries

Winery	Fusel oil, mg. per 100 ml. of wine				
	Angelica	Muscatel	Port	Tokay	Average
1	.....	17.7	26.6	18.3	20.9
2	26.4	.....	26.7	28.5	27.2
3	52.2	.....	53.2	44.8	50.1
4	.....	46.8	44.0	37.6	42.7

DISCUSSION

Since these wines constituted a good cross-section of California commercial wines, consideration was given to possible significance of the fusel oil content as a factor or criterion of quality. In each class, a committee of three judges at the State Fair had made awards for quality to about 3 to 6 entries, and these results were available including the tasting notes of this author who served on one of the four committees. The only table wine, a Zinfandel, which this writer had noted as having an odor suggestitng higher alcohols did actually contain the highest quantity found (41.7), although several other samples contained nearly as much. The angelica wine having a content of 90 mg. per 100 ml. was correctly described during the tasting as having excessive fusel oil and was eliminated for this reason. No other wines of any type contained more than approximately 55 mg. per 100 ml.

For a few of the larger classes an attempt was made to correlate the fusel oil content with the award-winning

TABLE 5

Comparison of fusel oil content of wines found by various investigators

Investigator	Number of wines	Type of wine	Fusel oil in mg.					
			Per 100 ml. of wine			Per 100 ml. of ethyl alcohol		
			Minimum	Maximum	Mean	Minimum	Maximum	Mean
Windisch *(10)*	14	White	16	68	35	153	611	353
Pettigiani *(9)*	24	Unknown	15	30	....	100	300	.....
Cioffi *(2)*	12	White	44	137	87	395	1230	729
	22	Red	14	172	77	86	1300	592
Guymon	120	White	16.2	36.6	25.0	131	308	208
	130	Red	14.0	41.7	28.7	142	344	235
	117	Dessert	15.6	90	37.4	78	486	202

wines but without success. Too many other factors affecting quality are involved in wines such as were studied here. Nevertheless, it is likely that the alcohols which are residues of the attack of amino acids by yeasts play an important role in the aroma of wines. Genevois *(4)* suggests the flavor importance of compounds such as phenylethyl alcohol and tyrosol which can result from the action of yeast upon phenylalanine and tyrosine, respectively.

The results found in this survey are summarized in Table 5 in comparison with those of other investigators, all calculated to the same basis. Agreement is best with the values of Pettigiani, who employed the colorimetric Penniman *(8)* procedure, and used a mixture of 4 g. of isoamyl alcohol and 1 g. of isobutyl alcohol as the standard of comparison. Minimum values of higher alcohols found are substantially the same as Windisch reported, but maximum values are considerably less than those of Windisch. The latter used the Rose method which depends upon the increase in volume of chloroform when it is used under rigidly defined conditions, to extract a solution of the alcohols.

Cioffi's values appear particularly high, especially in maximum readings. The explanation for his high average figures may be in the method of determination, for they appear sufficiently great as to materially affect the taste character of wines. Cioffi used a special adaptation of the Komarowsky reaction, employing salicylaldehyde as the color-producing reagent.

The intensity of color produced by the Komarowsky reaction from different higher alcohols depends on the choice of conditions. Isobutyl alcohol can give much more, equal, or less color than isoamyl, depending upon the conditions. Unless conditions suitable for equal color response from all the higher alcohols present can be found, which is unlikely, the quantity of fusel oil found in a particular wine by analysis would depend on the quantitative distribution of the several higher alcohols and the choice of a standard for comparison. However, at about 520 mμ isoamyl and isobutyl alcohols, at least, give nearly the same intensity of color under the analytical conditions described herein.

SUMMARY

The fusel oil content of 367 California commercial wines was determined by a colorimetric procedure based on the Komarowsky-Penniman method. The mean fusel oil content found for 120 white table wines was 25 mg. per 100 ml. of wine, ranging from a minimum value of 16.2 to a maximum of 36.6. For 130 red table wines, the average was 28.7, with range of variation 14 to 41.7. In 117 dessert wines, the average content of higher alcohols was 37.4, varying from 15.6 to 90.

Corresponding mean values expressed as mg. of higher alcohols per 100 ml. of ethyl alcohol were 208, 235, and 202, respectively, for the white table, red table, and dessert wines.

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Experimental Preservation of Flesh Foods with Antibiotics[a,b]

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The preservation of fish and meat with streptomycin, penicillin, subtilin, polymixin B, circulin, neomycin, bacitracin, gramicidin, metholyl gramicidin, tyrothricin, rimocidin, terramycin, chloromycin, aureomycin, and one unnamed antibiotic has been investigated. Aureomycin, terramycin, and chloromycetin, in the order named, proved the most effective inhibitors of growth of the natural mixed bacterial flora at temperatures between 0° C. and 21° C., while rimocidin inhibited yeast growth. Aureomycin caused marked inhibition of spoilage in 0.5 to 2.0 γ per g. concentration when incorporated in minced flesh, and was equally effective when applied by immersing steaks in solutions containing the antibiotic in 5 or 10 γ per ml. concentration. The remaining twelve antibiotics in 10 to 50 γ per g. concentration either exerted a less intense bacteriostatic action or were without effect.

One definition of the term antibiotic is, "a chemical compound derived from or produced by living organisms, which is capable, in small concentrations, of inhibiting the life processes of microorganisms" *(3)*. The early application of antibiotics was in medicine, but during the past two or three years their use has been extended to include animal feeding, for they frequently occasion a pronounced growth stimulus when included in rations of certain young domestic mammals and birds. The possibility of employing antibiotics to preserve human foods from undesirable bacterial attack has been recognized for several years, but there has as yet been no apparent practical application, and research on the subject has been rather limited.

Experiments with penicillic acid *(9)*, and later with streptomycin and penicillin *(11)*, indicated that they had a negligible or feeble bacteriostatic action in fish flesh stored at 0°C. Ground meat, inoculated with various *Clostridia* and incubated at 37°C. anaerobically was preserved fairly effectively for 5 or 10 day periods *(6)* with 20 γ per g. of a mixture of subtilin and streptomycin. In the same work it was reported that subtilin, penicillin, or streptomycin alone were less effective than the above mixture, and that tyrocidin and chloromycetin had little preservative action. Other investigators *(1)* reported that growth of strict anaerobes in comminuted beef was inhibited by only high concentrations of subtilin (100 or 1000 γ per g.), and that the effect of this antibiotic was probably transient. It has been shown that milk can be temporarily preserved against bacterial spoilage by penicillin *(5)* and that certain plant extracts, which possibly should not be termed antibiotics, act as bacteriostats in curing brines *(7)*. The possibility that processing temperatures and times in the canning of vegetables could be reduced if subtilin were used as a bacteriostat has been recognized *(2)*. However, further work has indicated that, though antibiotics may yet be of some value for this purpose, subtilin is not likely to prove reliable *(4)*.

It would seem that the use of antibiotics in non-canned foods offers more promise than does their addition to foods with the object of preventing bacterial spoilage of the canned product: in the former a transient bacteriostatic action may often suffice, while in the latter permanent inhibition of bacterial growth is usually mandatory. The present work is concerned with the first-named problem, and a note concerning the early results has been published *(12)*.

EXPERIMENTAL METHODS

Flesh of strictly fresh fish was obtained under conditions designed to minimize bacterial contamination. The beef used was obtained from freshly-slaughtered animals and was handled under similar conditions. Two methods of testing the various antibiotics were employed. In the first the flesh was passed through a domestic mincer equipped with a plate with 4 mm. diameter holes, and 95-g. portions were placed in 8-ounce sterilized screw capped glass jars. The antibiotic solutions were prepared immediately prior to use either as aqueous solutions, or, in the case of tyrothricin, gramicidin, and metholyl gramicidin, as solutions in 50% ethanol. Then 5-ml. portions of solu-

TABLE 1
Effect of antibiotics on growth of bacteria in halibut flesh at 0° C. (initial pH 6.2)

Antibiotic	Concentration	Bacteria × 10^6 per g. after		
		6 days	10 days	18 days
	γ per g.			
None	0	38.0	1600.0	....[c]
None	0	44.0	950.0	....[c]
Aureomycin hydrochloride	50	0.01	0.08	1300[d]
Aureomycin hydrochloride	25	0.01	0.35	1500[d]
Chloromycetin	50	0.10	79.0	3700[c]
Chloromycetin	25	0.24	230.0	....[c]
Subtilin	50	30.0	720.0	....[c]
Subtilin	25	20.0	680.0	....[c]
Sodium nitrite	200	0.10	44.0	....[c]

[c] Putrid. [d] All cells were yeasts.

TABLE 2
Effect of antibiotics on growth of bacteria in red spring salmon flesh at 3° C. (initial pH 6.2)

Antibiotic	Concentration	Bacteria × 10^6 per g. after	
		7 days	12 days[e]
	γ per g.		
None	0	32.0	3300
Aureomycin hydrochloride	50	0.0	0.27
Aureomycin hydrochloride	25	0.023	0.75
Aureomycin hydrochloride	10	0.0	3.2[f]
Chloromycetin	50	0.09	530
Chloromycetin	25	1.3	1400
Chloromycetin	10	3.7	4000
Subtilin	50	12.0	3900
Subtilin	25	31.0	3200
Sodium nitrite	200	0.08	20

[e] All samples except that treated with sodium nitrite showed severe pigment bleaching and rancidity.
[f] All cells were yeasts.

[a] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Mich., June 9, 1952.

[b] (EDITOR'S NOTE: The physiological effects of various antibiotics, if used in foods, has not been clearly established. Practical use of these agents in foods is not permitted at the present time.)

tions of the required concentration were incorporated into the 95-g. samples of flesh and the containers stored at the desired temperature. The second method of application merely consisted in immersing steaks of the required size for 1 minute in an aqueous solution of the antibiotic at about 10° C., draining for a short time to remove surplus liquid, wrapping in moisture-proof cellophane, and storing.

Comparative rates of microbiological spoilage of stored samples were determined as follows. A 25-g. portion was blended with 75 ml. of distilled water for about 1 minute, and two 0.1-ml. amounts were used to make duplicate preparations for a direct microbiological count *(10)* using North's staining procedure *(8)*. This counting method is rapid, yields results similar to those obtained by viable methods, gives relatively permanent preparations and permits an approximate differentiation of bacteria from yeast cells. However, it is rather inaccurate when bacterial populations of less than 1×10^5 per g. are involved *(10)*.

RESULTS

In Tables 1 to 6 the results obtained in experiments with minced flesh of different fish are recorded. In the first four experiments sodium nitrite was included because it has found extensive use in Canada in fresh fish, where it is permitted in concentration not exceeding 200 γ per g. (The Food and Drugs Act, page 76, Ottawa, 1949). It will be seen from the tables that aureomycin, terramycin, and chloromycetin were the only really effective antibiotics, their effectiveness being

TABLE 3

Effect of antibiotics on growth of bacteria in brill flesh at 3° C. (initial pH 6.2)

Antibiotic	Concentration	Bacteria × 10^6 per g. after	
		7 days	11 days
	γ per g.		
None	0	9.8	1500
Aureomycin hydrochloride	25	0.023	0.6
Aureomycin hydrochloride	10	0.023	<0.1
Terramycin hydrochloride	25	<0.01	<0.1
Terramycin hydrochloride	10	0.023	0.8
Chloromycetin	50	0.04	300
Chloromycetin	25	<0.01	390
Chloromycetin	10	0.22	2900
Penicillin	50	2.9	1900
Penicillin	25	1.6	680
Streptomycin	50	1.2	64
Streptomycin	25	1.1	100
Streptomycin	10	2.4	510
Sodium nitrite	200	<0.01	200

TABLE 4

Effect of antibiotics on growth of bacteria in coho salmon flesh at 3° C. (initial pH 6.2)

Antibiotic	Concentration	Bacteria × 10^6 per g. after		
		7 days	11 days	15 days
	γ per g.			
None	0	4.0	4500	.....
Aureomycin hydrochloride	10	<0.01	0.066	6.9[a]
Aureomycin hydrochloride	5	<0.01	0.053	32
Aureomycin hydrochloride	2	<0.01	0.053	350
Terramycin hydrochloride	10	<0.01	4.2	550
Terramycin hydrochloride	5	0.026	190	3500
Terramycin hydrochloride	2	0.21	730	4700
Neomysin sulfate	50	0.90	2400	.....
Neomysin sulfate	25	0.82	2300	.....
Circulin sulfate	50	0.08	480	4900
Circulin sulfate	25	0.08	650	4300
Polymixin B	50	0.026	670	4200
Polymixin B	25	0.200	960	4500
Sodium nitrite	200	0.013	14	2300

[a] Some yeasts.

TABLE 5

Effect of antibiotics on growth of bacteria in lingcod muscle at 0° C. and 3 ° C. (initial pH 6.6)

Antibiotic	Concentration	Bacteria × 10^6 per g. after days at [1]					
		3° C.			0° C.		
		6	10	13	6	10	13
	γ per g.						
None	0	1300			12	1200	3000
Aureomycin	2	1.3	31Y 1.2B	64Y 4.3B	0.11	0.026Y 0.43B	1.6Y 0.5B
Aureomycin plus rimocidin	2 10	0.92	1.0B	0.5Y 8.0B	0.026	0.26	0.6B
Aureomycin plus rimocidin	2 2	0.38	4.0Y 0.4B	48.0Y 5.0B	0.026	0.30	0.2Y 0.3B
Rimocidin	10	[b]	.....	.....	16	870	2500
Rimocidin	50	[b]	.....	.....	14	900	2600
Tyrothricin	50	1300	.....	.....	3.2	90	3000
Tyrothricin	10	1200	.....	.....	14	600	2700
Gramicidin	50	51	.....	.....	0.64	5	16Y 15B
Gramicidin	10	640	.....	.....	15	1100	2900
Metholyl gramicidin	50	16	.....	.....	0.29	3.3	100
Metholyl gramicidin	10	1400	.....	.....	8.1	800	2600
Polymixin B	50	640	.....	.....	4.7	26	3400
Polymixin B	10	1100	.....	.....	7.6	18	2800
631-51R788	50	1300	.....	.....	10.2	270	600
631-51R788	10	1400	.....	.....	15	1100	3200
Penicillin plus Streptomycin [1]	50	600	.....	.....	6.4	430	2300
Penicillin plus Streptomycin [1]	10	1000	.....	.....	10.2	1200	2900

[b] Putrid. [1] Equal quantities of each. [2] Y indicates yeast cells; B, bacteria.

TABLE 6

Effect of antibiotics in 10 γ per g. concentration on growth of bacteria in halibut muscle at 18-21° C. (initial pH 6.3)

Antibiotic [k]	Bacteria × 10^6 per g. after	
	1 day	2 days
None	10	4900 [l]
Aureomycin	<0.01	8 [m]
Aureomycin plus rimocidin	<0.026	4.9
Terramycin	0.13	12
Chloromycetin	0.15	360

[k] At this temperature neomycin, penicillin, streptomycin, polymixin B, bacitracin, gramicidin, tyrothicin, metholyl gramicidin, circulin, subtilin, equal amounts of penicillin and streptomycin, and rimocidin had only very feeble bacteriostatic action. Thus after 1 day the counts were between 1.1 and 2.7 × 10^6 per g., and all samples were putrid by the second day.
[l] Putrid. [m] Yeast cells.

in the order given. In some instances certain of the other antibiotics did exert a definite and quite pronounced bacteriostatic effect (gramicidin and metholyl gramicidin). This effect was never as marked as that obtained with much lower concentrations of aureomycin, and was not as consistent. Rimocidin was ineffective alone, but when used with aureomycin it retarded the yeast growth which usually occurred with samples stored for some days with aureomycin as bacteriostat. Sodium nitrite was very effective in the comparatively high concentration used.

TABLE 7

Effect of antibiotics on growth of bacteria in cooked crab meat at 3° C. (initial pH 7.9)

Antibiotic	Concentration	Bacteria × 10^6 per g. after	
		4 days	8 days
	γ per g.		
None	0	0.94	2700 [n]
None	0	1.2	2200 [n]
Aureomycin	20	<0.01	7.4 [p]
Aureomycin	10	<0.01	110 [p]
Aureomycin	5	0.053	320 [o]
Aureomycin	1	0.14	1300 [n]
Terramycin	20	<0.01	50 [p]
Terramycin	10	<0.01	280 [o]
Terramycin	5	<0.01	440 [o]
Terramycin	1	<0.01	630

[n] Putrid. [o] Stale odor. [p] Pleasant smell and taste.

The single experiment with cooked crab meat (Table 7) is of interest in that, with this rather alkaline material, aureomycin and terramycin gave about the same degree of protection against bacterial spoilage, while in all other experiments aureomycin proved more effective in a given concentration than did terramycin. Immersion of lingcod steaks in aureomycin solution (10 and 20 γ per ml.) caused a marked retardation of bacterial spoilage (Table 8). The bacteriostatic effect was so

TABLE 8

Effect of immersion in aureomycin solutions on growth of bacteria in lingcod steaks (initial pH 6.6; thickness about 2 cm.)

Solution used	Bacteria × 10^6 per g. at			
	3° C. after		0° C. after	
	6 days	9 days	13 days	23 days
Water	240	Putrid	330	Putrid
10 γ per ml. of aureomycin	<0.01	0.11	0.026	36
20 γ per ml. of aureomycin	<0.01	0.053	<0.01	12

marked that it would appear that dipping fish steaks in solutions containing even lower concentrations might prove satisfactory.

With minced beef only the three antibiotics which seemed of greatest interest in view of the foregoing experiments were investigated. The results (Table 9 and 10) indicated that as with fish, aureomycin is a somewhat more effective preservative for meat than is terramycin, but the differences were not as marked as those usually occurring with fish. In one series of experiments (Table 10) yeasts, and no noticeable bacteria, developed in the antibiotic-treated samples. Yeasts failed to develop in significant numbers in the other experiment in which rimocidin had been included in order to determine its effectiveness in inhibiting their growth. However, in the experiment in which beef steaks were dipped in aureomycin solutions (Table 11) rimocidin caused a very pronounced retarding effect on the yeast development which occurred with aureomycin alone.

TABLE 9

Effect of antibiotics on growth of bacteria in minced shoulder beef at different temperatures

Antibiotic	Concentration	Bacteria × 10^6 per g. at					
		0° C. after		4.5° C. after		18-21° C. after	
		8 days	23 days	4 days	8 days	1 day	2 days
	γ						
None	0	12	20,000 [q]	200	2,100	130	2,700
Aureomycin	1	0.1	1,500	0.66	280	0.01	300
Aureomycin	0.5	0.26	3,200	15	300	4.0	850
Aureomycin plus rimocidin	1.0 10.0	1.2	440	5.5	24.0	2.9	8.3
Aureomycin plus rimocidin	1.0 5.0	0.58	490	0.69	79	0.52	200
Terramycin	5.0	0.19	850	8.0	110	0.90	140
Terramycin	2.0	0.95	6,000	25	50	3.6	300
Terramycin plus rimocidin	2.0 10.0	14	20,000 [q]	37	860	8.0	3,100
Terramycin plus rimocidin	2.0 5.0	17	20,000 [q]	11	690	4.3	290

[q] These preparations were almost uncountable, and consequently the figures given are only rough approximations.

TABLE 10

Effect of antibiotics on growth of bacteria in minced beef shank meat at different temperatures

Antibiotic	Concentration	Bacteria × 10^6 per g. at 4.5° C. after 7 days	Bacteria × 10^6 per g. at 4.5° C. after 16 days	Bacteria × 10^6 per g. at 0° C. after 23 days
	γ per g.			
None	0	0.66	50,000 [a]	2,900
Aureomycin	10	<0.01	26Y [b]	5.8Y
Aureomycin	5	<0.01	58Y	4.6Y
Aureomycin	2	<0.01	37Y	6.6Y
Aureomycin	1	<0.01	58Y 90B	4.0Y
Terramycin	10	<0.01	95Y	9.0Y
Terramycin	5	<0.01	140Y 20B	3.7Y
Terramycin	2	.....	.....	720 B and Y

[a] Almost uncountable. [b] Y, indicates yeasts; B, bacteria.

TABLE 11

Effect of immersion in aureomycin and rimocidin solutions on growth of bacteria in round steaks stored at 4.5° C. for 12 days. (Thickness about 1.5 cm., weight roughly 300 g.)

Solution	Bacteria × 10^6 per g.
Water	2000
Aureomycin, 5 γ per ml.	5.8Y [c]
Aureomycin, 10 γ per ml.	6.5Y
Aureomycin, 20 γ per ml.	2.0Y
Aureomycin, 5 γ per ml. plus rimocidin, 10 γ per ml.	0.053Y
Aureomycin, 5 γ per ml. plus rimocidin, 20 γ per ml.	<0.01
Aureomycin, 10 γ per ml. plus rimocidin, 10 γ per ml.	<0.01
Aureomycin, 10 γ per ml. plus rimocidin, 20 γ per ml.	0.053Y

[c] Y, Yeast cells.

Acknowledgments

The kindness of the following companies in donating the antibiotics specified is gratefully acknowledged: Lederle Laboratories, aureomycin hydrochloride; Parke, Davis and Co., chloromycetin; Merck and Co. Inc., subtilin, sodium penicillin G-streptomycin calcium chloride mixtures, and antibiotic 631-51R788; The Upjohn Co., neomycin sulfate and circulin sulfate; S. B. Penick and Co., gramicidin, metholyl gramicidin, bacitracin, and tyrothricin; and Chas. Pfizer and Co. Inc., terramycin hydrochloride, rimocidin, polymixin B sulfate, potassium penicillin G and streptomycin sulfate.

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Filleting Fish and Freezing Fillets[a]

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A review covering the types of fish used for fillets together with the methods of preparing, freezing, and packaging fillets for market.

Fish filleting was first tried on an experimental basis by an operator on the Boston fish pier in 1921. The idea was intended to accomplish several results or overcome several difficulties common to the fish wholesalers at that time. Haddock were being landed in such quantities that there was little market for them. It was thought that if a boneless slice could be cut from each side of the fish, it would be much easier to market this slice than the whole fish. The depression of the market would be relieved and a demand for fish in this form would be created.

A fillet is a slice taken from each side of the fish; an expert can remove the slice including a maximum proportion of the edible meat and leave all the bones behind. The waste from filleting fish runs between 40 and 60% depending upon the species of fish being used for filleting. The remaining waste is used in various ways; the preparation of fish meal for animals and poultry feeding or in the preparation of cat and dog food. The sale of this material assists the producer to realize a monetary return on the entire fish; whereas, if the fish are sold round or eviscerated the waste material is scattered or ends in the family garbage can and brings no profit to anyone.

Most of the fillets are skinned, which adds to the attractiveness of them. There are machines which do this so that in many instances it has ceased to be a hand operation and thus does not add materially to the expense in the production of fillets.

Only a small percentage of the fillets are cut by machine. There is one machine which is successful for

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use in filleting cod and haddock; it does not handle other species of fish. This machine is located on the Boston, Massachusetts fish pier. Another similar machine has been perfected for filleting redfish or ocean perch. However, the research involved makes this type of study almost prohibitive because of the costs involved. Fish are so different in size and shape that it is difficult to design a machine which can take all shapes and sizes of fish and cut a fillet off either side and not include any bone with it.

Within the last month or two it is understood that a machine has been perfected by a San Francisco firm of engineers which will handle all species of fish. This machine also takes all sizes of the various species and cleans as well as fillets them. I have not seen one of these machines and cannot vouch for the value of the reports.

During the year 1951 there were approximatly 118 million pounds of fillets packaged and frozen. According to poundage, the species most often used was the Ocean Perch which made up 60% of the total fillets; the second most commonly used according to weight was the Haddock which made up approximately 17% of the total fillets frozen *(6)*.

When the fish are landed at the filleter's plant they are washed in fresh water and then passed through a mechanical scaler. The scaler is usually a rotating cylinder made of expanded steel mesh set at an angle so that the fish fed in at the high end are carried by gravity to the lower end. A high pressure water line passes through the axis of the cylinder. This water line is arranged so that a spray of water is played on the fish as they proceed to the lower end. When the fish have reached the lower end of the cylinder they are discharged onto a conveyor and are delivered to the filleting table or into a storage bin.

The arrangements of the filleting lines in the most of the fish plants are very similar. The filleters work at a long table which is fitted with a conveyor belt in the center and within easy reach of the person who is cutting the fillets. The table is fitted on the underside with a second conveyor to carry away the waste material. The cut fillets are usually placed in pans and are carried in the pans to the packagers. In some establishments another conveyor carries the cut fillets to the packers. There is some variation between plants depending upon the quantity of fish which is handled.

The filleters wear ordinary cotton work gloves when working; it enables them to hold the slippery fish with more ease. As the fish pass him on the conveyor he takes one and places it on the board in front of him with the head toward the left and with its back toward him. Holding the fish with his left hand usually, a thumb and finger in each eye, he cuts across the fish just back of the nape bone. He next slips the knife in the cut just made and slides the knife through to the tail along the back bone in one motion. Unless he has a razor sharp knife he will have trouble with the rib bones and some other small bones. He then turns the fish over and proceeds in a similar manner on the opposite side.

If the fillet is to be skinned it is placed skin side down on the board. The tail end is then pulled under the knife which is held parallel with the board with just enough pressure to slide between the skin and the flesh. There are a number of skinning machines in use which are more efficient and faster than the hand skinning.

The fillet is then placed in a pan or on a conveyor to be passed through a brine and chlorine bath and on to the packaging department. The object of the bath is cleansing the fillet and removal of any surface bacteria which may be present, also the brine causes a hardening of the cells and helps to promote a longer storage life with a reduction of "drip" upon defrosting *(1, 3)*. The presence of impurities in the salt in the form of calcium, magnesium salts and sulfates defeats the purpose of the brine treatment and causes "off" flavor and odors to be developed in storage. While the brining solution is made by a different formula in each plant, in general it contains from 3 to 15% salt and a chlorine concentration of 3 to 5 p.p.m. The fillets remain in the brine for periods varying from 20 to 120 seconds.

Fish of the flat species, soles and flounders, are handled in a slightly different method as are some of the Pacific Rock Fish. The former are cut the same way except that each fillet is split down the middle of the fish, making two fillets from each side of the fish. The skin is removed only on the side of the fish which shows color; the under side of the flat fish is white and the skin is seldom removed from the fillet prepared from that side. The Pacific Rock Fish group usually produce fillets which are quite thick. Directly under the skin is a layer of fatty tissue which if not removed causes the fillets to become rancid very rapidly when placed in cold storage *(5)*. It has been recommended that the fillets of this species be split making a fillet from the outer side and one from the inner side. When this is done a better keeping quality fillet can be prepared from the inside fillet, while the outside fillet can be sold unfrozen for immediate consumption.

The fillets are drained for a few minutes and then placed in packages for freezing. The size of the package depends upon the market the fillets are intended to reach. The individual consumer or family size package is usually 12 to 16 ounces in weight. The institutional package is large enough to contain 5 or 10 pounds of fillets. Usually the fillets going into the larger size packages are wrapped individually; those going into the smaller packages one pound or less, are wrapped as a unit. The cartons are usually of waxed cardboard; some of them have a window and are also over-wrapped. In most establishments the fillets are placed in an inner wrap of some moisture, vaporproof material and are then placed in the waxed carton. Then the carton is over-wrapped; this is usually handled by machine and is heat sealed.

In a process used by one filleting firm the fillets are placed in a trough measuring 4 by ¾ inches. The trough passes under rollers which press them into a sort of mold. This trough is made in sections of eight inches and the pressed fillets are cut off at that length. This block of fish 4 inches wide, 8 inches long and ¾ inch thick drops into a waxed carton as it is cut off. The package is then wrapped and heat sealed for freezing. The advantage of this package is that it can be cut in

the home so that each piece is the same size and the same thickness. All parts of this piece can be cooked to the same degree which is impossible with the ordinary fillet since the thick end will be under-cooked while the thin end will be over-cooked.

After the cartons of fillets are packed they are placed in trays or shallow pans for freezing. There are a number of freezing set ups, some operators make use of the plate frosters; others make use of the air blast *(2, 4)*. There is also the "sharp freezer" which is used in some plants. When the packages are frozen, they are finally packed in 50 pound cartons and sealed for storage or shipment.

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The Effect of Homogenization and Comminution on Cake Batters[a]

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The effect of homogenization and comminution of both completely and incompletely mixed cake batters on the quality of 5 different types of cakes has been investigated and reported.

Although the process of homogenization is widely used throughout the food industry, limited data have been published relating to its application in the baking industry and its effect on bread and cake. Glabau *(5)*, Carlin *(2)*, Bradley and Faulstich *(1)*, and Edelmann and Cathcart *(4)* have reported on the effect of homogenization on bread. MacDonnell, Hanson, Silva, Lineweaver, and Feeney *(8)* reported that homogenized liquid egg whites required additional whipping time and that cakes made with freshly homogenized egg whites had lower volumes than control cakes. They found that during storage at 36° F. (2° C.) for 3 to 7 days, the homogenized egg whites regained their original whipping properties.

The investigation reported in this paper was concerned with the effect of homogenization on completely mixed cake batters and also on slurries (incompletely mixed cake batters) containing all the cake ingredients. Because the high speed comminution of foods subjects them to powerful shearing forces similar to those employed in homogenization, comparisons were made during this investigation on the effect of comminution and homogenization on cake batters and slurries of cake ingredients.

In recent years at least three different machines, the Marco *(6)*, Votator *(3)*, and Oakes *(9)*, designed to mix cake batter in a continuous flow, have been introduced to the baking industry. During this investigation the practicality of using the homogenizer and the comminuting machine as continuous flow mixers was borne in mind and the authors were interested in learning whether these two machines, because of their more severe action on cake batter, would yield better cakes than those obtained with the Marco, Votator, or Oakes machine.

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EXPERIMENTAL METHODS

Description of equipment. The homogenizer used in this work was a laboratory-type high pressure Manton Gaulin machine Model No. 25-GGB. With this type machine, the mixture to be homogenized is forced through a fine orifice at various pressures. In this work pressures of 1000 and 3000 p.s.i.g. were used.

The comminuting machine or hammer mill is a device used in the food industry for chopping or pulverizing various ingredients. The comminuting machine used in this work was a Fitzpatrick Model D Fitzmill. The hammers consist of a set of 16 blades mounted on a shaft. These blades have a concave sharpened edge for chopping and a serrated blunt edge for pulverizing. A screen is inserted directly beneath the blades so that the material after passing through the cutting or pulverizing action is forced through the screen. In this work the pulverizing action and a No. 2 screen having circular openings 1/16 inch in diameter were used exclusively. Comminution was accomplished at the speed of approximately 5000 r.p.m.

Baking was done in an electrically heated Despatch Oven, Style No. 152-R, having automatic temperature controls. The oven had a rotating metal plate hearth and top and bottom heating elements.

Cake batters. The effect of homogenization and comminution on 5 different type cake batters was studied. Two, sponge and angel food, were foam-type batters. The others, yellow layer, gold pound, and white layer, were denser type batters. All batters when mixed in the regular manner were made on the Hobart Model A-200 Mixer. Yellow layer, gold pound, and white layer batters were mixed with a flat beater. The foam-type batters, sponge and angel food, were mixed with a wire whip. In this investigation all 5 cake batters were mixed in the regular manner. Portions of these batters were baked immediately after mixing and these cakes served as controls. Other portions of the regularly mixed yellow layer, gold pound, white layer, and sponge batters were homogenized at 1000 and 3000 p.s.i.g. and

the remaining portions of these batters were comminuted. Other portions of yellow layer and angel food batters mixed in the regular manner, were put through the homogenizer with the valves of the machine open (atmospheric pressure). This processing which includes the homogenization or comminution of regularly mixed batters is designated modifications "A" in Table 1.

It was of interest to study the effect of homogenization and comminution on slurries containing all the cake ingredients. This is reported for all batters except angel food. All ingredients were placed in the mixing bowl and stirred by hand just until the dry ingredients were wetted. Portions of this slurry were then homogenized at 1000 and 3000 p.s.i.g. and other portions were comminuted. This processing of slurries of all ingredients is designated modification "B" in Table 1.

A third modification, "C" in Table 1, was made with yellow layer and angel food batters. With yellow layer, all of the ingredients except the flour and baking powder were stirred together by hand and portions of the resultant slurry were homogenized at 1000 and 3000 p.s.i.g. and also with the homogenizing valves open. Another portion of the slurry was comminuted. After homogenization or comminution the flour and baking powder was folded in by hand. The same modification was made with angel food except that acid-calcium phosphate instead of baking powder was incorporated with the flour at the last stage of mixing.

RESULTS

In Table 2 are given the results of baking tests of yellow layer batters mixed according to the modifications described in Table 1. None of the yellow layer cakes made from homogenized or comminuted batters equaled the quality of the control. However, batters homogenized at 1000 and 3000 p.s.i.g. in "B" modification produced the best yellow layer cakes obtained from homogenized or comminuted batters. These were cakes in which all the ingredients had been mixed by hand until the dry ingredients were wetted and then the resultant slurry homogenized. They had good symmetry and grain but were lower in volume than the control and had a slightly tough crumb. These cakes had much better grain than the regularly mixed batters which had been homogenized at 1000 and 3000 p.s.i.g. Yellow layer batters to which the flour and baking powder were added after homogenization (modification "C") were tough with poor grain and texture. Comminuting yellow layer batters of "A," "B," and "C" modifications resulted in tough cakes with poor grain. The yellow layer cakes obtained with modifications "A," "B," and "C" and the control are illustrated in Figure 1.

In Table 2 are given results of baking tests of angel food batters mixed in the regular manner (control) and homogenized and comminuted as described in modifications "A" and "C" in Table 1. Slurries of all the angel food ingredients (modification B) were homogenized and comminuted but the resultant cakes were so poor that they were not scored. Angel food cakes obtained from modified batters were inferior to the control in all factors. Both homogenization and comminution of angel food batter resulted in extremely poor cakes.

When gold pound cake batter was processed as described in modifications "A" and "B" in Table 1, the resultant cakes were all inferior in quality to the control. Gold pound cakes made from modified batters cracked severely along the top surface. In addition,

TABLE 1
Modifications in processing tests

Modification [b]	Yellow Layer	Gold Pound	White Layer	Sponge Layer	Angel Food
Control	Baked immediately after mixing				
A	Homogenized atmospheric pressure [c] 1000, 3000 p.s.i.g.; also comminuted	Homogenized 1000, 3000 p.s.i.g.; also comminuted	Homogenized 1000, 3000 p.s.i.g.; also comminuted	Homogenized 1000, 3000 p.s.i.g.; also comminuted	Homogenized atmospheric pressure [c]
B	Homogenized 1000, 3000 p.s.i.g.; also comminuted	Homogenized 1000, 3000 p.s.i.g.; also comminuted	Homogenized 1000, 3000 p.s.i.g.; also comminuted	Homogenized 1000, 3000 p.s.i.g.; also comminuted	.....
C	Homogenized atmospheric pressure [c] 1000, 3000 p.s.i.g.; also comminuted	.....	.....	.....	Homogenized atmospheric pressure [c] 1000, 3000 p.s.i.g.; also comminuted

[b] Described in the text. [c] Homogenizing valve open.

TABLE 2
Scoring results for yellow layers and angel food cakes

	Control	Modification A				Modification B			Modification C			
		Homogenized p.s.i.g.			Comminuted	Homogenized p.s.i.g.		Comminuted	Homogenized p.s.i.g.			Comminuted
		Atmospheric	1000	3000		1000	3000		Atmospheric	1000	3000	
Yellow layers												
Volume, cc.	830	460	460	570	495	530	605	490	420	320	460	655
Symmetry	Good	Fair	Fair	Fair	Poor	Good	Good	Fair	Fair	Poor	Good	Good
Grain	Good	Poor	Poor	Poor	Poor	Good	Good	Poor	Poor	Poor	Poor	Poor
Texture	Good	Poor	Poor	Fair	Poor	Fair	Fair	Poor	Poor	Poor	Poor	Poor
Eating quality	Tender	Tough	Tough	Slightly tough	Tough	Slightly tough	Slightly tough	Tough	Tough	Tough	Tough	Tough
Angel food												
Symmetry	Good	Poor	.....	.....	.....	.....	.....	.....	Poor	Poor	Poor	Very poor
Grain	Good	Good	.....	.....	.....	.....	.....	.....	Poor	Poor	Poor	Very poor
Texture	Good	Poor	.....	.....	.....	.....	.....	.....	Poor	Poor	Poor	Very poor
Eating quality	Tender	Tough	.....	.....	.....	.....	.....	.....	Tough	Tough	Tough	Very poor

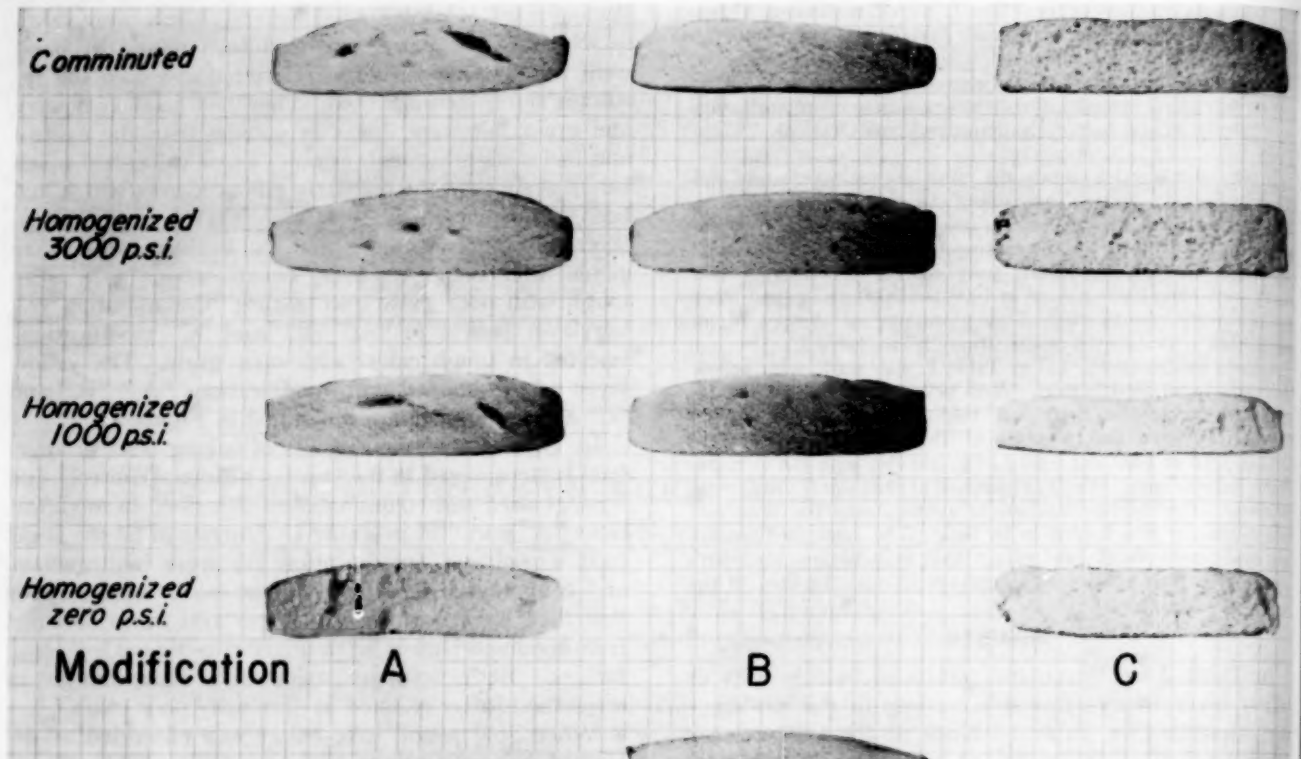

Figure 1. Yellow layer cake made from regularly mixed batter (control); from portions of the same batter after homogenization or comminution (A); from an homogenized or comminuted slurry of all ingredients (B); and from a slurry to which flour and baking powder were added after the other ingredients had been homogenized or comminuted (C).

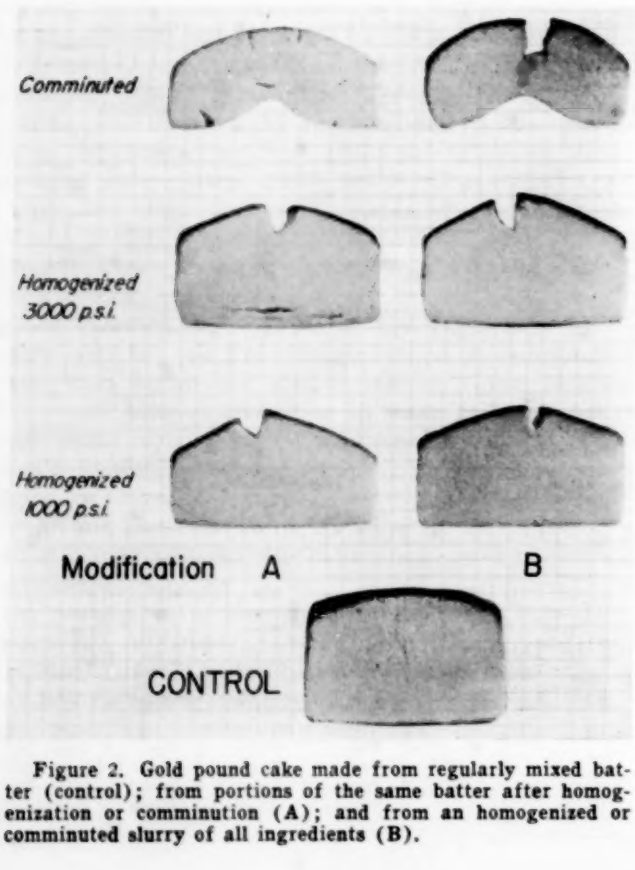

Figure 2. Gold pound cake made from regularly mixed batter (control); from portions of the same batter after homogenization or comminution (A); and from an homogenized or comminuted slurry of all ingredients (B).

cakes made from comminuted batters were warped and had inverted V shaped bottoms. Scoring results for these cakes are given in Table 3 and the cakes are illustrated in Figure 2. Although cake volume was not determined, inspection of Figure 2 shows the control to be of greater volume than the other cakes. The gold pound cake batters in which all ingredients had been mixed by hand until wetted (modification "B") and then homogenized at 1000 and 3000 p.s.i.g. yielded the best of the homogenized or comminuted batter cakes.

When white layer batter was processed as described in modifications "A" and "B" in Table 1, the resultant cakes were inferior to the control. Scoring results for these cakes are given in Table 3. Because results with white layer batters are similar to those obtained with yellow layer batter in modifications "A" and "B," the white layer cakes are not illustrated.

Sponge layer batters, processed as described in modifications "A" and "B" in Table 1, yielded cakes inferior to the control in all factors. Scoring details are given in Table 3 and the cakes are illustrated in Figure 3. Homogenizing or comminuting this foam-type batter resulted in misshapen, tough, dense cakes entirely different from the porous crumb structure of the control.

DISCUSSION

Homogenizing or comminuting five different types of cake batters yielded cakes inferior especially in eating quality, to the respective cakes mixed according to estab-

TABLE 3

Scoring results for gold pound, white layers, and sponge layer cakes

	Control	Modification A			Modification B		
		Homogenized p.s.i.g.		Comminuted	Homogenized p.s.i.g.		Comminuted
		1000	3000		1000	3000	
Gold pound							
Symmetry	Good	Poor	Poor	Very poor	Poor	Poor	Very poor
Grain	Good	Poor	Poor	Poor	Fair	Good	Poor
Texture	Good	Poor	Poor	Poor	Fair	Fair	Poor
Eating quality	Tender	Slightly tough	Slightly tough	Tough	Slightly tough	Slightly tough	Tough
White layer							
Volume, cc.	710	450	535	435	590	525	485
Symmetry	Good	Fair	Fair	Poor	Fair	Fair	Fair
Grain	Good	Poor	Fair	Poor	Poor	Poor	Poor
Texture	Good	Fair	Fair	Poor	Fair	Fair	Poor
Eating quality	Tender	Slightly gummy	Slightly gummy	Tough	Slightly tough	Slightly tough	Tough
Sponge layer							
Volume, cc.	960	200	380	220	290	330	195
Symmetry	Good	Poor	Poor	Poor	Poor	Poor	Poor
Grain	Good	Poor	Poor	Poor	Poor	Poor	Poor
Texture	Good	Poor	Poor	Poor	Poor	Poor	Poor
Eating quality	Tender	Tough	Tough	Tough	Tough	Tough	Tough

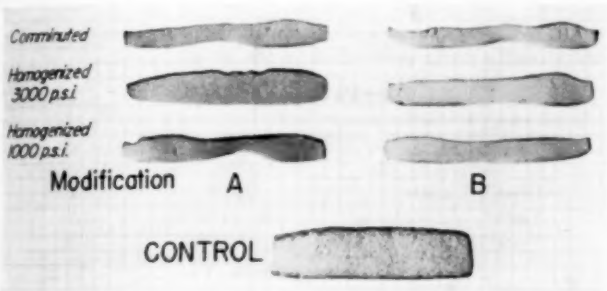

Figure 3. Sponge layer cake made from regularly mixed batter (control); from portions of the same batter after homogenization or comminution (A); and from an homogenized or comminuted slurry of all ingredients (B).

lished procedures. In this work the comminution of cake batters was more damaging to cake quality than homogenization. Differences between cakes made from modified and control batters were greater in sponge and angel food than in those made from denser batters. The quality and acceptability of cakes from the foam-type batters, sponge and angel food, was destroyed by either homogenization or comminution, while some cakes obtained from denser type batters which had been homogenized were acceptable.

Experience has shown that continuous flow mixers such as the Oakes machine successfully produce certain cakes made from foam-type batters. In the work reported here the quality of cakes made from foam-type batter suffered from homogenization. It is probable that the reasons for these differences in results are to be found in the fundamental differences in the design of the machines. A machine such as the Oakes is essentially a colloid mill while the Manton Gaulin machine is strictly a homogenizer. Although both machines produce emulsions, the colloid mill incorporates considerable air into the mixing during emulsification. The homogenizer on the other hand creates a fine dispersion while keeping the occlusion of air at a minimum. Hood and Lowe *(7)* have stated that air-evacuated batters showed little increase in cake volume and cakes made from such batters were considered unpalatable. It is likely that the essential difference in design accounts for the failure of the homogenizer to produce acceptable foam-type cakes and the relative success of the colloid mill type machine in producing such cakes.

SUMMARY

Under the conditions used in this work the homogenizer and comminuting machine were unsuccessful in producing the best quality cakes. However, with yellow layer type cakes it would appear that with some modifications these machines could be used. It appears from this work that lack of a means of incorporating air is a definite handicap.

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Flavor Quality and Strength of Propenyl Guaethol as a Vanilla Extender or Replacement[a]

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Organoleptic studies on the flavor strength and quality of propenyl guaethol were conducted. Comparisons were made with vanillin, ethyl vanillin, and coumarin, using pure vanilla extract as a standard of reference. Aroma and flavor profiles of a number of foods flavored with various combinations of these synthetics and pure vanilla extract were made. Propenyl guaethol was found to have 20 times the flavoring strength of vanillin. A blend of propenyl guaethol and vanillin most resembled pure vanilla extract in flavor quality.

Synthetic vanilla flavoring materials have been used in food products for many years. There is a constant search for flavoring materials which approach more closely the characteristics of pure vanilla extract, but which are more uniform in quality and less expensive than the natural product. The principal synthetic flavoring materials used for this purpose at present are vanillin, ethyl vanillin, and coumarin *(6)*. Heliotropin, often considered in this classification, is more widely used in industrial perfumes than in flavor compounds *(7)*. The object of our studies of propenyl guaethol was to determine the relative flavor quality of this material as a single flavoring agent and as an adjunct to other vanilla flavoring materials.

EXPERIMENTAL PROCEDURES

Aroma and flavor evaluation. Standard organoleptic panel methods were used for the determination of strength equivalents of the flavoring materials *(3)*, and for the profiles of flavored food products *(1, 2)*. Evaluation of all samples was made by three experienced judges selected for their high acuity in detecting small differences in vanilla-flavored food products *(4)*. Harrison, Ishler, and Laue *(5)*, have shown that inclusion of "poor" tasters weakens the mean ability of the panel. Replicate determinations by our small panel yielded less scattered and more reliable results than would have been obtained by a larger panel of less expert members.

The vanillin, ethyl vanillin, coumarin, and propenyl guaethol * used were fresh samples of commercial grade. The vanillin was made from eugenol. The pure vanilla extract used was a fresh sample of commercial standard vanilla extract of a brand previously selected by our panel as of excellent quality *(2)*.

Flavor equivalents were determined at detection-threshold level and identity-threshold level. Stock solutions of vanillin, ethyl vanillin, coumarin, and propenyl guaethol were prepared at a concentration of 1 g. per 100 ml. in 95% ethanol. These solutions and pure vanilla extract were progressively diluted with distilled water until detection-threshold levels were reached, as determined by duo-trio aroma and flavor evaluations. For each evaluation, the samples were compared with distilled water. The concentration of each compound in distilled water was progressively increased from the detection-threshold level to establish the identity-threshold level. Panel evaluation of these samples was conducted by the duo-trio technic and by direct comparison. Strength equivalents were determined at detection-threshold, identity-threshold, and reasonable-use levels.

Test foods. Profiles were established for sweetened water and sweetened milk flavored with the synthetics and with pure vanilla extract. Blends of the synthetics and pure vanilla were also used in sweetened milk and other foods for profile determination.

Sweetened water was selected as the prototype of vanilla-flavored foods in general, and sweetened milk as the prototype of vanilla-flavored foods which contain milk. The quantities of sugar and flavoring material added to milk and water were established by the panel as suitable for flavor profile studies and were within reasonable-use levels.

The formulations of the other test foods were selected as representative of the average commercial or household items. The amount of flavoring was adjusted to within the range of reasonable use, at a level most suitable for profile evaluation of each particular food.

In the following formulas, vanillin is shown as the flavoring material. For profile evaluations, each of the flavoring materials or combinations was used in the same formulas at equivalent strength levels established by the panel.

Formulas for test foods

Sweetened water	g.	% by weight
Distilled water	240.0	97.4
Sugar	6.45	2.6
Vanillin	0.042	0.017

Sweetened milk	g.	% by weight
Milk	240.0	97.4
Sugar	6.45	2.6
Vanillin	0.042	0.017

Hot chocolate	g.	% by weight
Water	45.0	17.4
Milk	195.0	75.2
Cocoa	6.6	2.5
Salt	0.17	0.07
Sugar	12.4	4.8
Vanillin	0.034	0.013

Puddings	g.	% by weight
Sugar	56.7	10.2
Starch	20.0	3.6
Carrageheen	0.5	0.1
Salt	0.6	0.11
Milk	480.0	86.0
Vanillin	0.2	0.036

Frostings	g.	% by weight
Butter	25.6	11.9
Confectioner's sugar	168.7	78.7
Salt	0.085	0.04
Milk	20.0	9.3
Vanillin	0.036	0.017

Layer cake	g.	% by weight
Eggs	33.7	7.6
Flour	120.3	27.1
Sugar	124.0	28.0
Shortening	45.6	10.3
Milk	120.0	27.0
Vanillin	0.044	0.010

[a] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Mich., June 11, 1952.

* The propenyl guaethol used was obtained from Shulton, Inc., Clifton, N. J., under the trade name, "Vanitrope." (Trade mark registered and patents pending.)

Aroma and flavor profiles. For our profile studies, aroma and flavor notes were established in preliminary sessions. These were: almond, floral, fruity, winey, phenolic, and dusty for aroma; and sweet, almond, woody, floral, fruity, phenolic, astringent, and bitter for flavor. Additional notes characteristic of particular test media were included as required.

Some of the aroma and flavor notes may require explanation as to meaning. In aroma, the floral and fruity notes are closely related, and together constitute the major "sweetness" of aroma. The almond note adds to "sweetness." The floral note suggests a combination of rose, orange blossom, lilac, and other sweet-scented flowers. The fruity note is also "sweet," but more pungent or acid, and has the general character of esters. The winey note suggests the general character of congeners in wine, not the grape note. The dusty note is similar to that of ordinary dust in the air.

In flavor, the sweet note is that of sugar. The woody note is vaguely related to the phenolic, but is more precisely that of an infusion of wood chips (not piney or resinous). It is also related to the winey note in the aroma. The floral and fruity notes are similar to those notes in the aroma.

For each of the test media, the sample flavored with pure vanilla extract was the reference standard, and its aroma and flavor were given overall ratings of 4.0. Aroma and flavor ratings of the other samples, established through a comparison of their various note strengths with those of the reference standard, show the relative resemblance of the various flavoring compositions to pure vanilla extract under the particular conditions of use indicated.

Note strength	Overall rating of closeness to pure vanilla
4.0 Very Strong	4.0 Excellent
3.5 Strong	3.5 Very Good
3.0 Predominant	3.0 Good
2.5 Slightly Predominant	2.5 Fair
2.0 Moderate	2.0 Fairly Poor
1.5 Weak	1.5 Poor
1.0 Detectable	1.0 Very Poor
0.5 Doubtful	
0.0 Absent	

Simmering of flavoring materials. In order to determine the relative stability of flavor strength and quality during cooking, each of the 4 synthetics and pure vanilla extract were simmered in distilled water solution. The concentrations of the flavoring materials simmered were double those used in sweetened water for flavor profile determinations, but still within the range of use levels. A 200-ml. portion of each solution in a 400-ml. beaker was heated at 90° C. in a forced-draft oven for 3.5 hours. During this time approximately 25% of the water was evaporated from each beaker. This water was replaced, then each simmered portion, and a corresponding unsimmered portion, were diluted with water. Sugar was added to the concentration previously established for profile determination in sweetened water. Profile determinations were then run by the panel.

RESULTS AND DISCUSSION

Threshold and equivalent strengths. Threshold and equivalent strength results, representing the panel consensus after several replicate determinations in each instance, are summarized in Table 1. These values have a reliability of plus or minus some 20 to 30%. Within this limit of accuracy, the aroma-detection threshold of each of the 5 flavoring materials was found to coincide with its flavor-detection threshold at the concentration in distilled water shown in Table 1. Similarly, the concentrations in distilled water at which pure vanilla extract, vanillin, and ethyl vanillin were identified by aroma coincided with those at which they were identified by flavor. However, vanillin and ethyl vanillin concentrations had to be raised to 20 times their detection-threshold levels to permit identification, due to their similarity in aroma and flavor notes, while pure vanilla extract was identified by its winey aroma and woody flavor at only 5 times its detection threshold. Coumarin was identified by aroma at only 5 times its detection level, and by flavor at 16 times detection level, largely due to its strong almond note. Propenyl guaethol was identified by flavor at 16 times detection level, but it had to be raised to 40 times detection level for aroma identity, because of its similarity to vanilla.

TABLE 1
Threshold and equivalent strengths of flavoring materials

	Standard pure vanilla extract	Vanillin	Ethyl vanillin	Coumarin	Propenyl guaethol
Detection threshold, p.p.m.	200	0.2	0.1	0.05	0.01
Equivalent strength	0.001	1	2	4	20
Identity threshold:					
Aroma, p.p.m.	1,000	4	2	0.25	0.4
Times detection level	5	20	20	5	40
Equivalent strength	0.004	1	2	16	10
Flavor, p.p.m.	1,000	4	2	0.8	0.16
Times detection level	5	20	20	16	16
Equivalent strength	0.004	1	2	5	25
Use level, p.p.m.	25,000	175	58	58	8.8
Times detection level	125	873	582	1164	873
Equivalent strength	0.007	1	3	3	20

The use-level concentrations given in Table 1 were selected, by panel consensus, after several replications of direct comparisons of aroma and flavor of a number of graded series of concentrations of each flavoring material in distilled water, sweetened water, and sweetened milk. They give substantially equivalent overall strengths of aroma and flavor for each of the materials in each of the media named, at least within the range of ± 30%. Although the level of flavoring selected, corresponding to 1.2 teaspoons of pure vanilla extract in 8 fluid ounces, is relatively high, it is still within the range of actual use in food products. This level was selected by the panel for profile determinations in order to accentuate differences in aroma and flavor note strengths. It should be kept in mind that this tends to lower the overall ratings of all of the synthetic flavoring materials, but a limited number of direct comparisons at lower flavoring levels indicated that such lowering is generally proportional. In other words, all of the synthetics approach pure vanilla extract in overall flavoring quality more closely at lower flavoring levels, but retain the same order of ratings found at the level given in Table 1. The panel also found that all of the materials retained the same overall equivalent strengths in each of the test media at concentrations down to one-fourth of the use levels given in Table 1, at least within ± 30%.

It will be noted that our panel confirmed the generally accepted equivalent strengths of ethyl vanillin and coumarin of 3 times that of vanillin in the use-level range. It is also generally accepted that a vanillin solu-

tion of 0.70% concentration is equivalent in flavoring strength to standard strength pure vanilla extract, and this was confirmed by our panel evaluation. On the same basis, in the use-level range, propenyl guaethol has 20 times the flavoring strength of vanillin.

Profiles of flavored and sweetened water and milk and effects of simmering. Aroma and flavor profiles of sweetened water and sweetened milk, flavored with each of the flavoring materials at the use levels given in Table 1, are shown in Table 2. The profiles in columns marked B are those of flavoring materials which were simmered in distilled water and evaluated after dilution and addition of the required amount of sugar.

None of the synthetics showed any detectable loss in strength or change in quality on simmering, except that propenyl guaethol showed considerable improvement in both aroma and flavor quality. It appears that simmering causes the phenolic note of the propenyl guaethol to shift to the winey-woody note so characteristic of pure vanilla extract. Pure vanilla extract suffered substantial loss in strength and change in quality on simmering.

Comparison of the profiles of flavored sweetened milk with those of sweetened water, unsimmered, shows that there is general improvement of the synthetics when evaluated in combination with milk. Generally, milk appears to suppress the dominant aroma and flavor notes of the flavoring materials. The winey-woody notes of pure vanilla extract are suppressed and the floral-fruity notes are balanced. The phenolic note of propenyl guaethol is suppressed. The characteristic almond note of coumarin is slightly suppressed. Vanillin, on the other hand, was very little improved by combination with milk because the dominant floral-fruity notes were not suppressed.

The closer similarity of the synthetics to pure vanilla extract when evaluated in combination with milk was an indication that the synthetics would even more nearly simulate the pure vanilla flavor in more complex foods. It will also be noted in Table 2 that ethyl vanillin most nearly resembles pure vanilla extract in aroma and flavor. Vanillin and propenyl guaethol follow next after ethyl vanillin in their resemblance to pure vanilla, although they have very different aroma and flavor characteristics. Coumarin, as the sole flavoring agent, bears little resemblance to pure vanilla extract. Because of the strong floral-fruity notes of vanillin and of the weak floral-fruity, but relatively stronger winey notes of propenyl guaethol, it was anticipated that a blend of these flavoring agents would produce a satisfactory vanilla flavor, probably more satisfactory than that of blends of ethyl vanillin and propenyl guaethol.

This was confirmed by preliminary studies of sweetened milk, through which the optimum blend of vanillin and propenyl guaethol was established as approximately 50:50 on a flavor-strength basis, or 20:1 on a weight basis. In fact, it developed that such a blend approached the flavor character of pure vanilla extract even more closely than expected from the individual profiles. This was apparently due to a synergistic effect whereby the character of the phenolic note of propenyl guaethol was shifted by the vanillin toward that of the winey-woody note of pure vanilla.

Blends of ethyl vanillin and propenyl guaethol were also investigated, and an optimum 50:50 flavor-strength blend found better than either alone, but not as similar to pure vanilla as the vanillin-propenyl guaethol blend. This appeared due not only to the lower floral-fruity note strength, but also to little if any synergistic effect of ethyl vanillin in shifting the phenolic note toward the winey-woody character of pure vanilla.

In a study of the relative aroma and flavor quality of flavored sweetened milk, in which increasing proportions of pure vanilla extract were replaced with equivalent proportions of vanillin, ethyl vanillin, and 50:50 vanillin-propenyl guaethol, it was found that replace-

TABLE 2

Aroma and flavor profiles of sweetened water and sweetened milk

	Pure vanilla extract			Vanillin			Ethyl vanillin			Coumarin			Propenyl guaethol		
	Water		Milk	Water		Milk	Water		Milk	Water		Milk	Water		Milk
	A[b]	B[c]		A	B		A	B		A	B		A	B	
Aroma															
Overall rating	4.0	1.3	4.0	2.7	2.7	2.7	3.0	3.0	3.4	0.6	0.6	1.2	1.7	2.4	2.7
Note strength															
Almond	1.0	1.0	0.5	0.5	0.5	0.5	1.0	1.0	0.5	4.0	4.0	3.5	1.0	1.0	1.0
Floral	2.5	1.0	2.0	2.5	2.5	2.5	3.5	3.5	2.0	3.0	3.0	2.0	1.5	2.0	1.5
Fruity	3.5	2.0	2.0	2.5	2.5	2.5	3.0	3.0	2.0	2.0	2.0	2.0	2.0	2.0	1.5
Winey	4.0	1.5	3.0	2.0	2.0	1.5	2.5	2.5	2.0	0.5	0.5	0.5	2.0	2.5	2.0
Phenolic	1.0	0.5	0.5	1.0	1.0	1.5	1.0	1.0	1.0	1.5	1.5	2.0	3.0	2.0	1.5
Dusty	1.0	1.0	0.5	1.0	1.0	0.5	1.0	1.0	0.5	1.0	1.0	0.5	1.0	1.0	0.5
Sour	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Milk	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0
Flavor															
Overall rating	4.0	1.4	4.0	2.3	2.3	2.4	2.7	2.7	3.3	0.4	0.4	1.4	2.2	2.4	2.9
Note strength															
Sweet	3.0	2.5	3.5	3.0	3.0	3.5	3.0	3.0	3.5	3.0	3.0	3.5	3.0	3.0	3.5
Almond	1.0	1.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	4.0	4.0	3.5	1.0	1.0	1.0
Woody	4.0	2.0	3.0	2.0	2.0	1.5	2.5	2.5	2.0	1.0	1.0	0.5	2.5	2.5	2.0
Floral	2.0	1.0	2.0	3.0	3.0	3.0	2.5	2.5	2.5	2.5	2.5	2.0	1.5	1.5	1.5
Fruity	3.0	1.5	2.0	3.0	3.0	3.0	2.5	2.5	2.5	1.5	1.5	2.0	2.0	2.0	2.0
Phenolic	1.0	1.5	1.0	1.5	1.5	1.0	1.5	1.5	1.0	2.0	2.0	1.5	3.0	2.5	2.0
Astringent	0.5	1.0	1.0	0.5	0.5	1.0	0.5	0.5	1.0	0.5	0.5	1.0	0.5	0.5	1.0
Bitter	1.5	1.5	1.0	2.0	2.0	2.0	1.5	1.5	1.0	2.0	2.0	1.5	1.5	1.5	1.0
Sour	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Milk	0.0	0.0	2.5	0.0	0.0	2.5	0.0	0.0	2.5	0.0	0.0	2.5	0.0	0.0	2.5

[b] Unsimmered. [c] Simmered.

ment of 25% of the vanilla with vanillin, 50% with ethyl vanillin, or 75% with 50:50 vanillin-propenyl guaethol, produced about equal, but very slight, overall departure from the flavoring quality of pure vanilla extract.

Profiles of food products. The relative flavor qualities of vanillin, ethyl vanillin, 50:50 vanillin-propenyl guaethol, and 50:50 ethyl vanillin-propenyl guaethol, as vanilla extenders, were studied through profile determinations of hot chocolate, frosting, and layer cake, flavored with each of these and with pure vanilla extract as a reference material. In order to accentuate differences in flavor quality for significant evaluation, these studies were made at 50% replacement of vanilla with vanillin, 75% replacement with ethyl vanillin, and 90% replacement with each of the propenyl guaethol blends. The results are given in Table 3.

Here it is seen that 90% replacement of vanilla with a 50:50 blend of vanillin-propenyl guaethol gives the best overall results, although not significantly better than 75% replacement with ethyl vanillin. Both resemble pure vanilla extract very closely in all these foods. In frosting, and even more in hot chocolate, 50% replacement of vanilla with vanillin gives significantly poorer results, and 90% replacement with 50:50 ethyl vanillin-propenyl guaethol gives still poorer results. However, both of these synthetic extenders make better showings in cake, although still slightly poorer than either ethyl vanillin or 50:50 vanillin-propenyl guaethol, at the replacement levels indicated. The higher ratings for all of the synthetic blends in cake are probably due, in part at least, to the effect of prolonged heating on the flavor quality of pure vanilla extract, the reference flavoring.

Since vanilla pudding desserts are now usually made in the home from prepared mixes, and these are generally flavored only with synthetics, we used no pure vanilla in the synthetic blends in this food for profile comparisons with pure vanilla extract. Flavoring materials used commercially in this product include principally vanillin or ethyl vanillin alone, blends of these, and blends of either or both with various proportions of coumarin. On the basis both of our experience in flavor evaluation of commercial pudding mixes and of preliminary studies by our panel, we selected a 49:49:2 blend of vanillin, ethyl vanillin, and coumarin as the optimum of this class for resemblance to pure vanilla extract in flavoring vanilla pudding. We compared this, by profile determinations, with 50:50 blends of propenyl guaethol with vanillin and ethyl vanillin in pudding, against pure vanilla extract. These results are given in Table 4, in which all flavoring material concentrations and proportions were, of course, in accordance with the flavor-strength equivalent established by the panel. (See Table 1.)

Here also the vanillin-propenyl guaethol blend resembles pure vanilla extract in both aroma and flavor much more closely than do either of the other synthetic blends. However, even the ethyl vanillin-propenyl guaethol blend, while significantly poorer, especially in aroma, than vanillin-propenyl guaethol, is more like pure vanilla extract than is the vanillin-ethyl vanillin-coumarin blend.

SUMMARY

Propenyl guaethol, a new vanilla-like synthetic, was compared with vanillin, ethyl vanillin, and coumarin for aroma and flavor quality and strength against pure vanilla extract as a standard, by an organoleptic panel.

TABLE 3

Aroma and flavor profiles of vanilla-flavored hot chocolate (H), frosting (F), and layer cake (C)

	Pure vanilla extract			Vanilla-vanillin			Vanilla-ethyl vanillin			Vanilla-propenyl guaethol-vanillin			Vanilla-propenyl guaethol-ethyl vanillin		
	100			50:50			25:75			10:45:45			10:45:45		
	H	F	C	H	F	C	H	F	C	H	F	C	H	F	C
Aroma															
Overall rating	4.0	4.0	4.0	2.9	3.3	3.5	3.6	3.6	3.8	3.6	3.8	3.8	2.5	3.1	3.6
Note strength															
Almond	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Floral	2.0	2.0	2.0	2.5	2.5	2.5	2.0	2.0	2.0	2.0	2.0	2.0	1.5	1.5	2.0
Fruity	2.0	2.0	2.0	2.5	2.5	2.5	2.0	2.0	2.0	2.0	2.0	2.0	1.5	1.5	2.0
Winey	3.0	3.0	2.0	1.5	2.0	1.5	2.0	2.0	1.5	2.5	2.5	1.5	1.5	2.0	1.5
Phenolic	0.5	0.5	0.5	1.0	0.5	0.5	0.5	0.5	0.5	1.0	0.5	0.5	2.0	1.0	1.0
Dusty	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chocolate	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0
Butter	0.0	1.5	0.0	0.0	1.5	0.0	0.0	1.5	0.0	0.0	1.5	0.0	0.0	1.5	0.0
Egg	0.0	0.0	1.5	0.0	0.0	1.5	0.0	0.0	1.5	0.0	0.0	1.5	0.0	0.0	1.5
Caramel	0.0	0.0	1.5	0.0	0.0	1.5	0.0	0.0	1.5	0.0	0.0	1.5	0.0	0.0	1.5
Flavor															
Overall rating	4.0	4.0	4.0	3.1	3.3	3.5	3.5	3.6	3.8	3.6	3.6	3.8	2.9	3.2	3.6
Note strength															
Sweet	3.0	4.0	3.5	3.0	4.0	3.5	3.0	4.0	3.5	3.0	4.0	3.5	3.0	4.0	3.5
Almond	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0	0.5	0.5
Woody	3.0	3.0	2.0	1.5	2.0	1.5	2.0	2.0	1.5	2.5	2.5	1.5	1.5	2.0	1.5
Floral	2.0	2.0	2.0	2.5	2.5	2.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Fruity	2.0	2.0	2.0	2.0	2.5	2.5	1.5	2.0	2.0	2.0	2.0	2.0	1.5	2.0	2.0
Phenolic	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.5	1.5	1.0	2.0	2.0	1.5
Astringent	1.0	0.5	0.5	1.0	0.5	0.5	1.0	0.5	0.5	1.0	0.5	0.5	1.0	0.5	0.5
Bitter	2.0	1.0	1.0	2.5	1.0	1.0	2.0	1.0	1.0	2.0	1.0	1.0	2.0	1.0	1.0
Chocolate	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0
Butter	0.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0	0.0
Egg	0.0	0.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0
Caramel	0.0	0.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0
Salt	0.5	0.5	1.0	0.5	0.5	1.0	0.5	0.5	1.0	0.5	0.5	1.0	0.5	0.5	1.0

TABLE 4
Aroma and flavor profiles of puddings

	Pure vanilla extract	Vanillin-ethyl vanillin-coumarin	Propenyl guaethol-vanillin	Propenyl guaethol-ethyl vanillin
	100	49:49:2	50:50	50:50
Aroma				
Overall rating	4.0	2.5	3.4	2.7
Note strength				
Almond	0.5	1.5	0.5	0.5
Floral	2.0	2.5	2.0	1.5
Fruity	2.0	2.5	2.0	1.5
Winey	3.0	1.5	2.0	1.5
Phenolic	0.5	1.0	1.0	1.5
Dusty	1.0	1.0	1.0	1.0
Milk	1.0	1.0	1.0	1.0
Flavor				
Overall rating	4.0	2.7	3.4	3.1
Note strength				
Sweet	3.5	3.5	3.5	3.5
Almond	0.5	1.5	0.5	0.5
Woody	3.0	1.5	2.0	2.0
Floral	2.0	2.5	2.0	2.0
Fruity	2.0	2.5	2.0	1.5
Phenolic	1.0	1.0	1.5	2.0
Astringent	1.0	1.0	1.0	1.0
Bitter	1.0	1.0	1.0	1.0
Milk	2.5	2.5	2.5	2.5
Salt	1.0	1.0	1.0	1.0

Concentrations and equivalent flavor strengths were determined by the panel at detection and identity thresholds and at a use-level deemed suitable for comparison of aroma and flavor qualities, by the profile method, in sweetened water and in sweetened milk. The effects of prolonged heating in water on each of the materials were also determined by the panel.

Blends of propenyl guaethol with vanillin and with ethyl vanillin were compared with vanillin and ethyl vanillin as extenders for pure vanilla extract in three representative food products, and with a blend of vanillin, ethyl vanillin, and coumarin as replacements for pure vanilla extract in pudding mix, by aroma and flavor profiles.

At use-level, propenyl guaethol was found to have 20 times the flavor strength of vanillin. The generally accepted flavor strengths, relative to vanillin, of 0.007 for standard vanilla extract and 3 each for ethyl vanillin and coumarin, were confirmed.

Prolonged heating in water resulted in substantial loss of aroma and flavor strength and quality of pure vanilla extract, but caused no significant change in strength or quality of any of the synthetics, except that the aroma and flavor quality of propenyl guaethol was slightly improved.

Of the four synthetics, used singly in sweetened water and in sweetened milk, ethyl vanillin most resembled pure vanilla, vanillin, and propenyl guaethol deviated about equally from pure vanilla, but in quite different aroma and flavor notes, while coumarin was quite unlike pure vanilla. The profiles of vanillin and propenyl guaethol indicated that a blend of the two would resemble pure vanilla more closely than any of the synthetics alone, and this was confirmed by profile studies.

The optimum blend of propenyl guaethol and vanillin was found to be equal parts on an equivalent strength basis (1:20, weight basis), and this blend was even more like pure vanilla than predicted from the profiles, apparently due to a synergistic effect. A similar blend of propenyl guaethol and ethyl vanillin gave nearly as good flavor results in layer cake, but not in other foods.

The optimum blend of propenyl guaethol and vanillin, used either as an extender or as a replacement for pure vanilla extract, in any of the representative foods studied, gave aroma and flavor qualities nearer those of pure vanilla than did any of the other synthetics, singly or in combinations.

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